

*Application  
for  
United States Letters Patent*

To all whom it may concern:

Be it known that      Richard Axel and Kristin Scott

have invented certain new and useful improvements in

CHEMOSENSORY GENE FAMILY ENCODING GUSTATORY AND ODORANT RECEPTORS  
AND USES THEREOF

of which the following is a full, clear and exact description.

**CHEMOSENSORY GENE FAMILY ENCODING GUSTATORY AND OLFACTORY  
RECEPTORS AND USES THEREOF**

5

This application claims the benefit of U.S. Provisional Application No. 60/271,319, filed February 23, 2001, the contents of which are hereby incorporated by reference.

10 The invention disclosed herein was made with Government support under grant numbers NS 29832-09 from the National Institutes of Health and 2POICA23767-22 from the National Cancer Institute. Accordingly, the U.S. Government has certain rights in this invention.

15

**Background Of The Invention**

20 Throughout this application, various publications are referenced in parentheses. Full citations for these references may be found at the end of the specification immediately preceding the claims. The disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains.

25

All animals have specialized mechanisms to recognize and respond to chemosensory information in the environment. Olfactory neurons recognize volatile cues that afford the organism the ability to detect food, predators and mates.

30 In contrast, gustatory neurons sense soluble chemical cues that elicit feeding behaviors. In insects, taste neurons also initiate innate sexual and reproductive responses. In *Drosophila*, for example, sweet compounds are recognized by chemosensory hairs on the proboscis and legs that activate

20084846 032002

proboscis extension and feeding (Dethier, 1976). Sexually dimorphic chemosensory bristles on the foreleg of males recognize cues from receptive females that are thought to elicit the embrace of mating (Tompkins et al., 1983; Possidente and Murphey, 1989). Females have yet a third set of specialized bristles on their genitalia that may cause oviposition in response to nutrients (Rice, 1977; Taylor, 1989). In this manner, gravid females will preferentially deposit their eggs on a rich environment that enhances survival of their offspring. These robust and innate gustatory responses provide the opportunity to understand how chemosensory information is recognized in the periphery and ultimately translated into specific behaviors.

Taste in *Drosophila* is mediated by sensory bristles that reside on the proboscis, legs, wing, and genitalia (Stocker, 1994; Singh, 1997). Most chemosensory bristles are innervated by four bipolar gustatory neurons and a single mechanoreceptor cell (Falk et al., 1976). The dendrites of gustatory neurons extend into the shaft of the bristle and are the site of taste recognition that translates the binding of tastants into alterations in membrane potential. The sensory axons from the proboscis project to the brain where they synapse on projection neurons within the subesophageal ganglion (SOG), the first relay station for gustatory information in the fly brain (Stocker and Schorderet, 1981; Nayak and Singh, 1983; Shanbhag and Singh, 1992; Rajashekhar and Singh, 1994). Sensory axons from taste neurons at other sites along the body project locally to peripheral ganglia (Power, 1948). *Drosophila* larvae, whose predominant activity is eating, sense their chemical environment with gustatory neurons that reside in chemosensory organs on the head and are

also distributed along the body surface (Stocker, 1994).  
The pattern of projection of functionally distinct classes  
of taste cells and therefore the nature of the  
representation of gustatory information in the *Drosophila*  
5 brain remains unknown.

The identification of the genes encoding taste receptors  
and the analysis of the patterns of receptor expression  
may provide insight into the logic of taste discrimination  
10 in the fly. In *Drosophila*, the recognition of odorants is  
thought to be accomplished by about 70 seven-transmembrane  
domain proteins encoded by the *Drosophila* odorant receptor  
(DOR) gene family (Clyne et al., 1999; Gao and Chess,  
1999; Vosshall et al., 1999; Vosshall et al., 2000).  
15 Recently, a large family of putative G protein-coupled  
receptors was identified by searching the *Drosophila*  
genome with an algorithm designed to detect seven-  
transmembrane domain proteins (Clyne et al., 2000). These  
genes were suggested to encode gustatory receptors (GRs)  
20 because members of this gene family were detected in the  
proboscis by RT-PCR experiments.

The present application characterizes and extends the  
family of putative G protein-coupled receptors originally  
25 identified by Clyne et al. (2000) and provides evidence  
that they encode both olfactory and gustatory receptors.  
In situ hybridization, along with transgene experiments,  
reveals that some receptors are expressed in  
topographically restricted sets of neurons in the  
30 proboscis, whereas other members are expressed in  
spatially fixed olfactory neurons in the antenna. Members  
of this gene family are also expressed in chemosensory  
bristles on the leg and in larval chemosensory organs.  
Finally, the projections of different subsets of larval

chemosensory neurons were traced to the subesophageal ganglion and the antennal lobe. These data provide insight into the diversity of chemosensory recognition in the periphery and afford an initial view of the representation of gustatory information in the fly brain.

10081815-022202

# Summary of the Invention

This invention provides an isolated nucleic acid encoding an insect gustatory receptor protein, wherein the receptor protein comprises seven transmembrane domains and a C-terminal domain, and the C-terminal domain comprises consecutive amino acids having the following sequence:

-G-L/F-F-X-X-X-X-X-X-X-X-X-X-X-X-X-T-Y-L-V/I-L-V/I/L-Q-F- (SEQ ID NO: 60),

where X is any amino acid, and / means or.

The invention provides an isolated nucleic acid encoding an insect odorant receptor protein, wherein the receptor protein comprises seven transmembrane domains and a C-terminal domain, and the C-terminal domain comprises consecutive amino acids having the following sequence:

-G-L/F-F-X-X-X-X-X-X-X-X-X-X-X-X-X-T-Y-L-V/I-L-V/I/L-Q-F- (SEQ ID NO: 60),

where X is any amino acid, and / means or.

The invention provides an isolated nucleic acid encoding an insect gustatory receptor protein, wherein the nucleic acid molecule encodes a protein selected from the group consisting of:

- (a) an insect receptor protein comprising consecutive amino acids having a sequence

identical to that set forth for Gr2B1 in SEQ ID  
NO: 1,

5 (b) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr8D1 in SEQ ID  
NO: 2,

10 (c) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr10B1 in SEQ ID  
NO: 3,

15 (d) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr10B2 in SEQ ID  
NO: 4,

20 (e) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr28A2 in SEQ ID  
NO: 5,

25 (f) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr28A4 in SEQ ID  
NO: 6,

30 (g) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr33C1 in SEQ ID  
NO: 7,

(h) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr36B2 in SEQ ID NO: 8,

5

(i) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr36B3 in SEQ ID NO: 9,

10

(j) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr59C1 in SEQ ID NO: 10,

15

(k) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr61D1 in SEQ ID NO: 11,

20

(l) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr63F1 in SEQ ID NO: 12,

25

(m) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr64A2 in SEQ ID NO: 13,

30

(n) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for GR64A3 in SEQ ID NO: 14,



- 5 (o) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr66C1 in SEQ ID  
NO: 15,
- 10 (p) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr92D1 in SEQ ID  
NO: 16,
- 15 (q) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr98A1 in SEQ ID  
NO: 17,
- 20 (r) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr98A2 in SEQ ID  
NO: 18,
- 25 (s) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr2940.1 in SEQ  
ID NO: 19,
- 30 (t) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr2940.2 in SEQ  
ID NO: 20,
- (u) an insect receptor protein comprising  
consecutive amino acids having a sequence

identical to that set forth for Gr2940.3 in SEQ  
ID NO: 21,

5 (v) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr2940.4 in SEQ  
ID NO: 22,

10 (w) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr2940.5 in SEQ  
ID NO: 23,

15 (x) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr57B1 in SEQ ID  
NO: 46,

20 (y) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr93F1 in SEQ ID  
NO: 48,

25 (z) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr93F2 in SEQ ID  
NO: 49,

30 (aa) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr93F3 in SEQ ID  
NO: 50,

20230927

(bb) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr93F4 in SEQ ID NO: 51,

5

(cc) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr94E1 in SEQ ID NO: 52,

10

(dd) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr93D1 in SEQ ID NO: 53,

15

(ee) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for GrLU1=Gr36B1 in SEQ ID NO: 55,

20

(ff) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for GrLU2=Gr28A3 in SEQ ID NO: 56,

25

(gg) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for GrLU3=Gr64A1 in SEQ ID NO: 57,

30

(hh) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for GrLU7=Gr5A1 in SEQ ID NO: 59, and

(ii) an insect gustatory receptor protein which shares from 7-50% amino acid identity with any one of the proteins of (a)-(hh), and comprises seven transmembrane domains and a C-terminal domain, wherein the C-terminal domain comprises consecutive amino acids having the following sequence:

-G-L/F-F-X-X-X-X-X-X-X-X-X-X-X-X-X-T-Y-L-V/I-L-V/I/L-Q-F- (SEQ ID NO: 60),

where X is any amino acid, and / means or.

The invention provides an isolated nucleic acid molecule encoding an insect odorant receptor protein, wherein the nucleic acid molecule encodes a protein selected from the group consisting of:

(a) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr2B1 in SEQ ID NO: 1,

(b) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr8D1 in SEQ ID NO: 2,

(c) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr10B1 in SEQ ID NO: 3,

(d) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr10B2 in SEQ ID NO: 4,

5

(e) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr28A2 in SEQ ID NO: 5,

10

(f) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr28A4 in SEQ ID NO: 6,

15

(g) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr33C1 in SEQ ID NO: 7,

20

(h) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr36B2 in SEQ ID NO: 8,

25

(i) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr36B3 in SEQ ID NO: 9,

30

(j) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr59C1 in SEQ ID NO: 10,

- 5 (k) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr61D1 in SEQ ID NO: 11,
- 10 (l) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr63F1 in SEQ ID NO: 12,
- 15 (m) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr64A2 in SEQ ID NO: 13,
- 20 (n) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for GR64A3 in SEQ ID NO: 14,
- 25 (o) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr66C1 in SEQ ID NO: 15,
- 30 (p) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr92D1 in SEQ ID NO: 16,
- (q) an insect receptor protein comprising consecutive amino acids having a sequence

identical to that set forth for Gr98A1 in SEQ ID  
NO: 17,

5 (r) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr98A2 in SEQ ID  
NO: 18,

10 (s) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr2940.1 in SEQ  
ID NO: 19,

15 (t) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr2940.2 in SEQ  
ID NO: 20,

20 (u) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr2940.3 in SEQ  
ID NO: 21,

25 (v) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr2940.4 in SEQ  
ID NO: 22,

30 (w) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr2940.5 in SEQ  
ID NO: 23,

(x) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr57B1 in SEQ ID NO: 46,

5

(y) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr93F1 in SEQ ID NO: 48,

10

(z) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr93F2 in SEQ ID NO: 49,

15

(aa) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr93F3 in SEQ ID NO: 50,

20

(bb) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr93F4 in SEQ ID NO: 51,

25

(cc) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr94E1 in SEQ ID NO: 52,

30

(dd) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr93D1 in SEQ ID NO: 53,



- 5 (ee) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for GrLU1=Gr36B1 in SEQ ID NO: 55,
- 10 (ff) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for GrLU2=Gr28A3 in SEQ ID NO: 56,
- 15 (gg) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for GrLU3=Gr64A1 in SEQ ID NO: 57,
- 20 (hh) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for GrLU7=Gr5A1 in SEQ ID NO: 59, and
- 25 (ii) an insect odorant receptor protein which shares from 7-50% amino acid identity with any one of the proteins of (a)-(hh), and comprises seven transmembrane domains and a C-terminal domain, wherein the C-terminal domain comprises consecutive amino acids having the following sequence:
- 30 -G-L/F-F-X-X-X-X-X-X-X-X-X-X-X-T-Y-L-V/I-L-V/I/L-Q-F- (SEQ ID NO: 60),
- 35 where X is any amino acid, and / means or.

The invention provides a nucleic acid molecule comprising at least 12 nucleotides which specifically hybridizes with any of the isolated nucleic acid molecules described  
5 herein.

This invention provides a vector which comprises any of the isolated nucleic acid molecules described herein.

- 10 The invention provides a host vector system for production of a polypeptide having the biological activity of an insect gustatory or odorant receptor, which comprises any of the vectors described herein and a suitable host.
- 15 The invention provides a method of producing a polypeptide having the biological activity of an insect gustatory or odorant receptor which comprising growing any of the host vector systems described herein under conditions permitting production of the polypeptide and recovering  
20 the polypeptide so produced.

The invention provides a purified insect gustatory or odorant receptor protein encoded by any of the isolated nucleic acid molecules described herein.  
25

- The invention provides an antibody which specifically binds to an insect gustatory or odorant receptor protein encoded by any of the isolated nucleic acid molecules described herein. The invention provides an antibody  
30 which competitively inhibits the binding of any of the antibodies described herein capable of specifically binding to an insect gustatory or odorant receptor.

The invention provides a method of transforming a cell which comprises transfecting a host cell with any of the vectors described herein.

- 5 The invention provides a transformed cell produced by any of the methods described herein.

10 The invention provides a method of identifying a compound which specifically binds to an insect gustatory or odorant receptor which comprises contacting any of the transformed cells described herein, or a membrane fraction from said cells, with the compound under conditions permitting binding of the compound to the gustatory or odorant receptor, detecting the presence of any such compound  
15 specifically bound to the receptor, and thereby identifying the compound as a compound which specifically binds to an insect gustatory or odorant receptor.

20 The invention provides a method of identifying a compound which specifically binds to an insect gustatory or odorant receptor which comprises contacting any of the purified insect gustatory or odorant receptor proteins described herein with the compound under conditions permitting binding of the compound to the purified gustatory or  
25 odorant receptor protein, detecting the presence of any such compound specifically bound to the receptor, and thereby identifying the compound as a compound which specifically binds to an insect gustatory or odorant receptor.

30

The invention provides a method of identifying a compound which activates an insect gustatory or odorant receptor which comprises contacting any of the transformed cells described herein, or a membrane fraction from said cells,

with the compound under conditions permitting activation of the gustatory or odorant receptor, detecting activation of the receptor, and thereby identifying the compound as a compound which activates an insect gustatory or odorant  
5 receptor.

The invention provides a method of identifying a compound which activates an insect gustatory or odorant receptor which comprises contacting any of the purified insect  
10 gustatory or odorant receptor proteins described herein with the compound under conditions permitting activation of the gustatory or odorant receptor, detecting activation of the receptor, and thereby identify the compound as a compound which activates an insect gustatory or odorant  
15 receptor.

The invention provides a method of identifying a compound which inhibits the activity of an insect gustatory or odorant receptor which comprises contacting any of the  
20 transformed cells described herein, or a membrane fraction from said cells, with the compound under conditions permitting inhibition of the activity of the gustatory or odorant receptor, detecting inhibition of the activity of the receptor, and thereby identifying the compound as a  
25 compound which inhibits the activity of an insect gustatory or odorant receptor.

The invention provides a method of identifying a compound which inhibits the activity of an insect gustatory or  
30 odorant receptor which comprises contacting any of the purified insect gustatory or odorant receptor proteins described herein with the compound under conditions permitting inhibition of the activity of the gustatory or odorant receptor, detecting inhibition of the activity of

the receptor, and thereby identifying the compound as a compound which inhibits the activity of an insect gustatory or odorant receptor.

- 5 The invention provides a compound identified by any of the methods described herein.

The invention provides a method of combating ingestion of crops by pest insects which comprises identifying a  
10 compound by any of the methods described herein and spraying the crops with the compound.

The invention provides a method of controlling a pest population in an area which comprises identifying a  
15 compound any of the methods described herein and spraying the area with the compound.

The invention provides a composition which comprises a compound identified by any of the methods described herein  
20 and a carrier.

The invention provides a method of preparing a composition which comprises identifying a compound by any of the methods described herein, recovering the compound from the  
25 receptor protein, and admixing a carrier.

20220194.022007

# Brief Description Of The Figures

**Figure 1A-1B.** The signature motif of GRs is present but diverged in members of the DOR gene family.

5 Sequence alignments of the complete DOR and GR gene families reveal a common amino acid motif in the putative seventh transmembrane domain of the carboxyl terminus of all GRs and 33 DORs. Alignments are shown for 23 GRs and 33 DORs (from top to bottom of figure, SEQ ID NO: 61  
10 through SEQ ID NO: 116, respectively). The average identity in the C-terminus is 29% for the GRs, 25% for the DORs, and 20% for the GRs plus DORs. Sequence relationships between the GR gene family and the DOR genes were analyzed with HMMs (Eddy, 1998), CLUSTAL alignments  
15 and neighbor joining trees (Saitou and Nei, 1987; Higgins and Sharp, 1988), and NxN BLASTP (Rubin et al., 2000) comparisons. The consensus alignment and coloring of conserved residues was assigned in ClustalX.

20 **Figure 2A-2B.** Expression of GR genes in the proboscis and antenna

Digoxigenin-labeled antisense riboprobes derived from GR sequences hybridize to subsets of cells in adult chemosensory organs. (A) Six genes show specific  
25 hybridization to gustatory tissues. *Gr47A1*, *Gr66C1*, *Gr32D1*, *Gr98A1*, *Gr28A3* and *Gr33C1* are expressed in single cells within chemosensory sensilla of the proboscis labellum (data not shown for *Gr28A3* and *Gr33C1*). (B) Three genes, *Gr63F1*, *Gr10B1*, and *Gr21D1*, are specifically  
30 detected in the medial aspect of the third antennal segment, the adult olfactory organ. These expression patterns were maintained in more than 50 heads for each riboprobe. Probes were annealed to sagittal sections (15

um) of the adult fly head to assay for expression in the proboscis and to frontal sections to examine expression in the antenna.

5 **Figure 3. A spatial map of GR expression in the proboscis**

GR promoter-Gal4 transgenes drive expression in subsets of cells in the proboscis. Flies containing GR promoter-Gal4 and UAS-lacZ transgenes were examined for B-galactosidase activity staining on labial palp whole mounts. Each labial  
10 palp contains 31-36 chemosensory sensilla, arranged in approximately four rows. In the diagram of a labial palp, different rows of sensilla are depicted in different colors (adapted from Ray et al., 1993). Individual GRs show restricted expression in discrete subsets of  
15 chemosensilla. Gr47A1 is expressed in 9-11 sensilla innervating the most peripheral row of bristles, Gr32D1 is expressed in 6 sensilla innervating an intermediate row of bristles, Gr22B1 is expressed in only 3-4 sensilla innervating small bristles, and Gr66C1 and Gr28A3 are  
20 expressed in 8-10 sensilla innervating small or medium bristles. The spatial patterns for the different receptors are identical in 2-5 independent transformant lines for each promoter construct, and are also fixed among over 20 different individuals within a line.

25

**Figure 4A-4E. GRs are expressed in a variety of chemosensory neurons**

(A, B) Expression of GFP allows visualization of dendrites and axons of neurons in the proboscis. GFP was  
30 detected in labial palp whole mounts of GR promoter-Gal4: UAS-GFP flies by direct fluorescence microscopy. Each transgene drives expression of GFP in a single bipolar neuron within a sensillum. Gr66C1 is expressed in 9 neurons (6-7 in focus) (A) and Gr22B1 is expressed in 3

neurons (B) innervating different rows of chemosensory bristles.

(C, D, E) GRs are expressed in chemosensory sensilla that reside on the internal mouthparts of the proboscis and on  
 5 tarsal segments of legs. In addition to expression in the proboscis labellum, *Gr32D1*, *Gr66C1* and *Gr28A3* are also detected in the cibarial organs of the mouth. (C) *LacZ* expression in a whole mount proboscis is illustrated for the *Gr66C1-Gal4: UAS-lacZ* line. The arrow denotes the  
 10 cibarial organ. (D) One transgenic line, *Gr2B1-Gal4*, drives expression exclusively in the labral sense organ of the mouth, and not in the cibarial organs or in the labellum of the proboscis. The arrow denotes the labral sense organ. (E) *Gr32D1* is expressed in the proboscis  
 15 labellum and in the cibarial organs. In addition, *Gr32D1-Gal4* drives expression of GFP in 2-3 neurons in the fourth and fifth tarsal segments of all legs. Receptor expression was examined by B-galactosidase activity staining of *GR promoter-Gal4: UAS-lacZ* flies (C, D) or by  
 20 fluorescent visualization of *GR promoter-Gal4: UAS-GFP* flies (E).

**Figure 5A-5G. GRs are expressed in larval chemosensory neurons**

25 (A) The antenno-maxillary complex of larvae is a bilaterally symmetric structure containing the dorsal organ mediating smell and the terminal organ involved in both taste and smell. Shown is the anterior ventral region of a larva viewed by differential interference  
 30 contrast. On one half of the larval head, the sensilla of the terminal organ is outlined with black dotted lines and the pore of the terminal organ is denoted by an outlined arrow. The dome of the dorsal organ is denoted by a filled arrowhead.



(B-E) *Gr32D1*, *Gr66C1*, and *Gr28A3* are expressed in the proboscis labellum in the adult (Figure 3), and are expressed in a single bilaterally symmetric neuron in the terminal organ of larvae (B, E, data not shown). *Gr2B1* is  
 5 expressed in the labral sense organ of the adult proboscis, and is expressed in two neurons innervating the dorsal organ (filled arrow), one neuron innervating the terminal organ (outlined arrow), and one neuron innervating the ventral pits in each of the thoracic  
 10 segments in larvae (C). *Gr21D1* is expressed in the adult antenna and in a single larval neuron innervating the terminal organ (D). The dome of the dorsal organ is autofluorescent.

(F,G) Different GRs are expressed in distinct  
 15 chemosensory neurons. In larvae bearing two *GR promoter-Gal4* fusions and *UAS-GFP*, two GFP positive cells per terminal organ are observed. The different promoter combinations illustrated are *Gr21D1-Gal4* plus *Gr66C1-Gal4* (F) and *Gr32D1-Gal4* plus *Gr66C1-Gal4* (G). The  
 20 pseudotracheae of the larval mouth shows autofluorescence.

#### Figure 6A-6H. Axonal Projections of Larval Chemosensory Neurons

Projections of neurons bearing different GRs are spatially  
 25 segregated in the larval brain. In all panels, whole mount larval brains from *GR promoter-Gal4: UAS-nSyb-GFP* flies were stained with anti-GFP to label axonal termini (green), mAb nc82 to label neuropil (red), and TOTO-3 to counterstain nuclei (blue). Each image represents a  
 30 composite of 1  $\mu$ m optical sections through the larval brain, encompassing the terminal projections. Projections extend 5-10  $\mu$ m in depth for B,C,D,G and 10-20  $\mu$ m in depth for E,F,G.

(A) The larval brain is composed of the two dorsal brain

hemispheres (BH) and the ventral hindbrain (HB). The subesophageal ganglion (SOG) resides in the hindbrain, at the juncture of the hindbrain with the brain hemispheres. The antennal lobe (AL) is a small neuropil on the anterior  
 5 edge of the brain hemisphere (denoted with an arrow in panel E,G).

(B-D) GR-bearing neurons project to discrete locations in the larval brain. *Gr32D1* is expressed in the proboscis in the adult and in one neuron in the terminal organ in  
 10 larvae. In *Gr32D1-Gal4:UAS-nSyb-GFP* larval brains, a single terminal arborization is observed in the SOG (C). A similar pattern is observed for neurons expressing *Gr66C1*, a gene expressed in the adult proboscis and in a single  
 15 neuron in the terminal organ and two in the mouth of larvae (B, D). Panels D is a higher magnification (3x) of Panel B.

(E) Projections of gustatory neurons from different body regions are spatially segregated in the fly brain. *Gr2B1* is expressed in two neurons innervating the dorsal organ,  
 20 one neuron innervating the terminal organ, and one neuron innervating the ventral pits. Axons from ventral pit neurons enter the hindbrain via thoracic nerves and terminate in the antennal lobe (arrows), in a location that is distinct from the termini of other *Gr2B1*-bearing  
 25 neurons.

(F) Segregation is less apparent in the terminal projections of two different taste receptors. Larvae that contain *Gr66C1-Gal4* and *Gr32D1-Gal4* along with *UAS-nSyb-GFP* reveal two partially overlapping projection patterns.

30 (G,H) Distinct projection patterns are observed for the two different chemosensory modalities, taste and smell. *Gr21D1* is expressed in the adult antenna and in a single neuron in the terminal organ of larvae. *Gr21D1* axons enter the antennal lobe (arrows) (G). In larvae that

contain *Gr21D1-Gal4* and *Gr66C1-Gal4* along with *UAS-nSyb-GFP*, two discrete termini are apparent, one entering the SOG, and a second entering the antennal lobe (H).

5 **Figure 7A-7C. A subset of GRs encode olfactory receptors**

- GR-bearing neurons in the antenna project to discrete glomeruli in the antennal lobe. Adult transgenic flies in which *Gr21D1 promoter-Gal4* drives expression of *UAS-lacZ* (A) or *UAS-GFP* (B) show specific labelling in subsets of
- 10 cells in the medial aspect of the antenna. This expression pattern resembles that determined for the endogenous gene. LacZ expression was detected in 15 um frontal sections of the antenna (A); GFP expression was examined in whole antennae (B).
- 15 (C) *Gr21D1*-bearing neurons project to a single bilaterally symmetric glomerulus on the ventral-most region of the antennal lobe. Whole mount brains of *Gr21D1-Gal4: UAS-nSyb-GFP* flies were examined by fluorescent immunohistochemistry, with anti-GFP to visualize axonal
- 20 termini of *Gr21D1*-bearing neurons (green), mAb nc82 to label brain neuropil (red), and TOTO-3 to counterstain nuclei (blue). *Gr21D1*-bearing neurons send projections to the V glomerus in the antennal lobe (Stocker et al., 1990; Laissue et al., 1999) and do not project to the
- 25 subesophageal ganglion (located in the bottom part of C).

10081916 033302

### Detailed Description Of The Invention

Throughout this application, the following standard  
 5 abbreviations are used to indicate specific amino acids:

3-character abbreviation	Amino Acid	1-character abbreviation
Ala	Alanine	A
Arg	Arginine	R
Asn	Asparagine	N
Asp	Aspartic Acid	D
Cys	Cysteine	C
Gln	Glutamine	Q
Glu	Glutamic Acid	E
Gly	Glycine	G
His	Histidine	H
Ile	Isoleucine	I
Leu	Leucine	L
Lys	Lysine	K
Met	Methionine	M
Phe	Phenylalanine	F
Pro	Proline	P
Ser	Serine	S
Thr	Threonine	T
Trp	Tryptophane	W
Tyr	Tyrosine	Y
Val	Valine	V
Asx	Asparagine/ Aspartic Acid	B
Glx	Glutamine/ Glutamic Acid	Z
***	(End)	*
Xxx	Unidentified, any, or as specified.	X

Throughout this application, the following standard  
 abbreviations are used to indicate specific nucleotides:

C=cytosine	A=adenosine
T=thymidine	G=guanosine.

This invention provides a family of isolated nucleic acid molecules encoding insect gustatory and odorant receptors. In one embodiment, the receptor is a gustatory receptor. In one embodiment, the receptor is an odorant receptor.

The family of receptors comprises:

Newly identified receptors disclosed herein comprise:

**Gr2B1** (SEQ ID NO: 1)

MDTLRALEPLHRACQVCNLPWRLAPPPDSEGILLRRSRWLELYGWTVLIAATSFTV  
YGLFQESSVEEKQDSESTISSIGHTVDFIQLVGMVAHLAALLEALWQRQAQRGFFA  
ELGEIDRLLSKALRVDVEAMRINMRRQTSRAVWILWGYAVSQLLILGAKLLSRGDR  
FPIYWISYLLPLLVCGLRYFQIFNATQLVRQRLDVLLVALQQQLQHKGPAVDTVLE  
EQEDLEEAAMDRLIAVRLVYQRVWALVALLNRCYGLSMLMQVGNDFLAITSNCYWMF  
LNFRQSAASPFDILQIVASGVWSAPHLGNVLVLSLLCDRTAQCASRLALCLHQVSVD  
LRNESHNALITQFSLQLLHQRLHFSAAGFFNVDCITLLYTIVGATTTYLIILIQFHMS  
ESTIGSDSNGQ

**Gr8D1** (SEQ ID NO: 2)

MSGHLGRVLQFHLRLYQVLGFHGLPLPGDGNPARTRRRRLMAWSLFLLSLALVLAC  
LFSGEEFLYRGDMFGCANDALKYVFAELGVLAIIYLETLSQRHLANFWWLHFKLGGQ  
KTGLVSLRSEFQQFCRYLIFLYAMMAEVAIHLGLWQFQALTQHMLLFWSTYEPLVW  
LTYLRNLQFVLHLELLREQLTGLEREMGLLAEYSRFASETGRSFPGFESFLRRRLVQ  
KQRIYSHVYDMLKCFQGA FNFSILAVLLTINIRIAVDCYFMYYSIYNNVINNDYYLI  
VPALLEIPAFIYASQSCMVVVPRIAHQLHNIIVTDSGCCSCPDLQLQIQLNFSLLHQ  
PIRIDCLGLTILDCSLLTRMACSVGTYMIYSIQFIPKFSNTYM

**Gr10B1** (SEQ ID NO: 3)

MORTHLEFEFKNAPQEPKRPFEFFMYFKFCLINLMMMIQVCGIFAQYGEVGKGSVSQ  
VRVHFAIYAFVLWNYTENMADYCYFINGSVLKYYRQFNLQLGSLRDEMDGLRPGGML  
LHHCCELSDRLEELRRRCREIHDLORESFRMHQFQLIGLMLSTLINNLTNFYTLFHM  
LAKQSLEEVSYPPVVVGSVYATGFYIDTYIVALINEHIKLELEAVALTMRRFAEPREM  
DERLTREVRNKFISFLATTLLEIMIQLIWSFFANFDDVTPYRKCNRPKNLFFKIRQK

VIGIVSSGKLKLLVSLRFFIIDNRLILNLHKYLAIKLNFLNLIQIEHLSLELLNYQP  
PMLCGLLHLDRRLVYLIAVTAFSYFITLVQFDLYLRKKS

**Gr10B2** (SEQ ID NO: 4)

MRVGKLCRLALRFWMGLILVLGFSSHYYNPTRRLVYSRILQTYDWLLMVINLGAFY  
LYYRYAMTYFLEGMFRRQGFVNQVSTCNVFQQLMAVTGTWLHFLFERHVCQTYNEL  
SRILKHDCLKKEHSRFYCLAFLAKVYNFFHNFNFALSAIMHWGLRPFNVWDLNLY  
FVYNLARDAILVAYVLLLLNLSEALRLNGQQEHDTYSDLMKQLRRRERLLRIGRRV  
HRMFAWLVAIALIYLVFFNTATIYLGYTMTFIQKHDALGLRGRGLKMLLTVVSFLVIL  
WDVLLQVICEKLLAEENKICDCPEDVASSRTTYRQWEMSALRRAITRSSPENNVLG  
MFRMDMRCAFALISCSLSYGI III IQIGYIPG

**Gr28A2** (SEQ ID NO: 5)

MAFKLWERFSQADNVFQALRPLTFISLLGLAPFRLNLNPRKEVQTSKFSFFAGIVHF  
LFFVLFCFGISVKEGDSIIGYFFQTNITRFS DGTLRLTGILAMSTIFGFAMFKRQRLV  
SIIQNNIVVDEIFVRLGMKLDYRRILLSSFLISLGMLLFNVIYLCVSYSLVVSATIS  
PSFVTFTTFALPHINISLMVFKFLCTTDLARSRFSMLNEILDILDAHIEQLSALEL  
SPMHSVNVNHRYSRHLRNLISTPMKRYSVTSVIRLNPEYAIKQVSNIHNLCDICQT  
IEEYFTYPLLGIIAISFLFILFDDFYILEAILNPKRLDVFEADEFFAFFLMQLIWI  
VIIVLIVEGSSRTLHSSYTAAIVHKILNITDDPELRDRLFRLSLQLSHRKVLFTAA  
GLFRLDRTLIFTVN FLQITGAATCYLIILIQF

**Gr28A4** (SEQ ID NO: 6)

MIRCGLDIFRGCRGRFRYWLSARDCYDSISLMVAIAFALGITPFLVRRNALGENSLEQ  
SWYGFLNAIFRWLLLAYCYSYINLRNESLIGYFMRNHVSQISTRVHDVGGIIAAVFTF  
ILPLLLRKYFLKSVKNMVQVDTQLERLRSPVNFNTVVGQVVLVILAVVLLDVTLLTTG  
LVCLAKMEVYASWQLTFIFVYELLAISITICMFCMLTRTVQRRITCLHKFDFATMSAL  
RRVRKYFISSQVYEALRPLFFLTFLYGLTPFHVVRKMGESYKMSCFGVFNIFIYIC  
LCGFCYISSLRQGESIVGYFFRTEISTIGDRLQIFNGLIAGAVIYTSAILKRCKLLGT  
LTIHSLDTNFSNIGVRVKYSRIFRYSLVLIFKLLILGVYFVGVFRLLVSLDVTPSF  
CVCMTFFLQ

**Gr33C1** (SEQ ID NO: 7)

MKRKAVEVIGLIPLNRQQSETNFILDYAMMCIVPIFYVACYLLINLSHIIIGLCLLDSC

NSVCKLSSHLFMHLGAFLYLTITLLSLYRRKEFFQQFDARLNDIDAVIQKCQORVAEMD  
KVKVTAVKHSVAYHFTWLFVFTFALYDVRSLYLTFGNLAFIPFMVSSFPYLAGS  
IIQGEFIYHVSVISQRFEQINMLLEKINQEARHRHAPLTVFDIESEGKKERKTVTPIT  
VMDGRTTTFGFGNENKFAGEMKRQEGQKNDDDDLDTSNDEDEDDFDYDNATIAENTGN  
TSEANLPDLFKLHDKILALSVITNGEFGPQCVPYMAACFVVSIFGIFLETKNVFIVGG  
KSRLLDYMTYLYVIWSFTTMMVAYIVLRLCCNANNHKSQSAMIVHEIMQKKPAFMLS  
DLFYNKMKSFTLQFLHWEGFFQFNGVGLFALDYTFIFSTVSAATSYLIVLLQFDMTAL  
LRNEGLMS

**Gr36B2** (SEQ ID NO: 8)

MVDWVVLKAVHIYCYLIGLSNFEFDCRTGRVFKSRRCTIYAFMANIFILITIIYNF  
TAHGDTNLLFQSANKLHEYVIIIMSGLKIVALITVLNRWLQRGQMMQLVKDVIRLYMI  
NPQLKSMIRWGILLKAFISFAIELLQVTLSDALDRQGTAEEMGMLLVKLCVSFIMNLA  
ISQHFLVILLIRAQYRIMNAKLRMVIEWESRRLSFLQLRNGAFMTRCCYLSQLEDIGE  
VQSQQLQSMVGQLDEVFGMQGLMAYSEYYLSIVGTSYMSYSIYKYGPHNLKLSAKTSII  
VCILITLFYLDALVNCNNMLRVLDHKKDFLGLLEERTVFASSLDIRLEESVSFESLQL  
QLARNPLKINVMGMFPITRGSTAAMCASVIVNSIFLIQFDME

**Gr36B3** (SEQ ID NO: 9)

MDLESFLLGAVYYYGLFIGLSNFEFDWNTGRVFTKKWSTLYAIALDSCIFALYIYHWT  
GNTNIVNAIFGRANMLHEYVVAILTGLRIVTGLFTLILRWYQRCKMMDLASKVVRMYV  
ARPQVRRMSRWGILTKFIFGSITDGLQAMVLSAMGSRVDSQFYLGLGLQYWMFVILN  
MAMMQQHMIIMLFVRTQFQLINTELRLQVIDEAKDLLSPRHQGVFMTKCCSLADQIENI  
ARIQSQLOTIMNQMEEVFGIQGAMTYGGYYLSSVGTCYLAISILKHGYENLSMTLSTV  
ILAYSWCFFYYLDGMLNLSVMLHVQDDYWEMLQILGKRTIFVGLDVRLEEAVST

**Gr59C1** (SEQ ID NO: 10)

MIKLYFRYSLAIGITSQQFSNRKFFSTLFSRTYALIANIVTLIMLPIMVWQVQLVFQQK  
KTFPKLILITNNVREAVSFLVILYTVLSRGFRDTAFKEMQPLLLTLFREEKRCGFKGIG  
GVRRSLRILLFVKFFTLWLCTDVLFLLYSTDALIWNVLRFFFKCNTNNILEMVPMG  
YFLALWHIARGFDCVNRRLDQIVKSKSTRKHRELQHLWLLHACLTKTALNINKIYAPQM  
LASRFDNFVNGVIQAYWGAVFTFDLSTPFFWVYGSVQYHVRCLDYILIDNMCDVAVEY  
HDSAKHSWSEVRWTKEVSAFGSILLYICMLMQLLSFQISSYVIYANSTKLQLWSCGLFQ  
ANRSMWFAMISSVLYIILVLLQFHLVMRK\*

**Gr61D1** (SEQ ID NO: 11)

MSRTSDDIRKHLKVRQRKQRAILAMRWRC AQGGLEFEQLDTFYGAIRPYLCVAQFFGIM  
 PLSNIRSRDPQDVKFKVRSIGLAVTGLFLLLGGMKTLVGANILFTEGLNAKNIVGLVFL  
 IVGMVNWLN FVGFARSWSHIMLPWSSVDILMLFPPYKRGKRSLSKVNVLALS VVVLAV  
 GDHMLYYASGYCSYSMHILQCHTNHSRITFGLYLEKEFS DIMFIMPFNIFSMCYGFWLN  
 GAFTFLWNFMDIFIVMTSIGLAQRFQQFAARVGALEGRHVPEALWYDIRRDHIRLCELA  
 SLVEASMSNIVFVSCANNVYVICNQALAIFTKLRHPINYVYFWYSLIFLLARTSLVMT  
 ASKIHDASLLPLRSLYLVPSDGWTQEVQRFADQLTSEFVGLSGYRLFCLTRKSLFGMLA  
 TLVTYELMLLQIDAKSHKGLRCA

**Gr63F1** (SEQ ID NO: 12)

MRPSGEKVVKGHGQGN SGHSLSGMANYYRRKKGDAVFLNAKPLNSANAQAYLYGVRKYS  
 IGLAERLDADYEAPPLDRKKSSDSTASNNEFKPSVFYRNIDPINWFLRIIGVLPIVRH  
 GPARAKFEMNSASFIYSVVFVLLACYVGYVANNRIHIVRSLSGPFEEAVIAYLFLVNI  
 LPIMIIPILWYEARKIAKLFNDWDDFEVLYYQISGHSLPLKLRQKAVYIAIVLPILSVL  
 SVVITHVTMSDLNINQVVPYCILDNL TAMLGAWWFLICEAMSI TAHLAERFQKALKHI  
 GPAAMVADYRVLWLRLSKLTRDTGNALCYTFVFM SLYLFFIITLSIYGLMSQLSEGFGI  
 KDIGLTITALWNIGLLFYICDEAHYASVNVRTNFQKKLLMVELNWMNSDAQTEINMFLR  
 ATEMNPSTINC GGFFDVNRTLFKGLLTTMVTYLVVLLQFQIS IPTDKGDSEGANNITVV  
 DFVMDSLDNDMSLMGASTLSTTTVGTTLPPPIMKLKGRKG

**Gr64A2** (SEQ ID NO: 13)

MPVRKVSSKFAEDLTFTWFSVRSYYALVTILFFGVSSGYMVAFVTSVSFNFD SVETLVF  
 YLSIFLISLSFFQLARKWPEIAQSWQLVEAKLPPLKLPKERRSLAQHINMITIVATTCS  
 LVEHIMSMLSMGYYVNSCPRWPDRPIDSFYLSFSSVFYFVDYTRFLGIVGKVVNLST  
 FAWNFNDIFVMAVSVALAARFRQLNDYMMREARLPTTVDYWMQCRINFRNLCKLCEEVD  
 DAISTITLLCFSNNLYFICGKILKSMQAKPSIWHALYFWFSLVYLLGRTLILSLYSSSI  
 NDESKRPLVIFRLVPREYWCDELKRFSEEVQMDNVALTGMKFFRLTRGVVISVAGTIVT  
 YELILLQFN GEEK

**Gr64A3** (SEQ ID NO: 14)

MELSRSDKEAFLSDGSFHQAVGRVLLVAEFFAMMPVKGV TGKHPSDLSFSWRNIRTCTF  
 SLLFIASSLANFGLSLFKVLNNPISFNSIKPIIFRGSVLLVLIVALNLARQWPQLMMY

20220919 10:04:00



WHTVEKDLPOYKTQLTKWKMIGHTISMVMMLGMMLSFAEHILSMVSAINYASFCNRTAD  
PIQNYFLRTNDEIFFVTSYSTTLALWGKFQNVFSTFIWNYMDLFVMIVSIGLASKFRQ  
LNDDLRFNFKGMNMAPSYWSERRIQYRNICILCDKMDDAISLITMVSFSNNLYFICVQLL  
RSLNTMPVAHAVYFYFSLIFLIGRTLAVSLYSSSVHDESRLTLRYLRCVPKESWCPEV  
KRFTEEVISDEVALTGMKFFHLTRKLVLSVAGTIVTYELVLIQFHEDNDLWDCDQSYYS

**Gr66C1** (SEQ ID NO: 15)

MDNMAQAEDAVQPLLQQFQQLFFISKIAGILPDLEKFRSRNLLEKSRNGMIYMLSTLI  
LYVVLNINILYISFGEEDRSLKASQSTLTFVIGLFLTYIGLIMMVSDQLTALRNQGRIGE  
LYERIRLVDERLYKEGCVMDNSTIGRRIRIMLIMTVIFELSILVSTYVKLVLDYSQWMSL  
LWIVSAIPTFINTLDKIWFAVSLYALKERFEAINATLEELVDTHEKHKLWLRGNQEVPP  
PLDSSQPPQYDSNLEYLYKELGAIDAASRKPPPPPLATNMVHESELGNAAKVEEKLNNL  
CQVHDEICEIGKALNELWSYPILSLMAYGFLIFTAQLYFLYCATQYQSIPSLFRSAKNP  
FITVIVLSYTSKGKCVYLIYLSWKTSQASKRTGISLHKCGVVADDNLLYEIVNHLCLKLL  
NHSVDFSACGFFTLDMETLYGVSGGITSYLIILIQFNLAQQAKEAIQTFNSLNDTAGL  
VGAATMDNISSTLRDFVTTTMTPAV

**Gr92D1** (SEQ ID NO: 16)

MFEFLHQMSAPKLSTSILRYIFRYAQFIGVIFFCFLHTRKDDKTVFIRNWLKWLNVTHRI  
ITFTRFFWVYIASISIKTNRVLQVLHGMRLVLSIPNAVILCYHIFRGPEIIDLINQFL  
RLFRQVSDLFKTKTPGFGGRRELILILLNLISFAHEQTYLWFTIRKGFSWRFLIDWWCD  
FYLVSATNIFIHINSIGYLSLGVLYSELNKYVYTNLRIQLQKLNTSGSKQKIRRVQNRL  
EKCISLYREIYHTSIMFHKLFVPLLFLALIYKVLLIALIGFNVAVEFYLN SFIFWILLG  
KHVLDLFLVTVSVEGAVNQFLNIGMQFGNVGDLSKFQTTVSQFIFIDFIPI

**Gr98A1** (SEQ ID NO: 17)

MVAQKSRLARAFPYLDIFSVFALTPPPQSFGHTPHRRLRWYLMTGIVFYATAILATVF  
IVSYFNIIAIDEEVLEYNVSDFTVRMGNIQKSLYSIMAIANHLNMLINRYRLGGIYKDI  
ADLEMDMDEASQCFGGQRQRFSSFRFRMALCVGVMMILMVGSMPRLTMTAMGPFVSTLLK  
ILTEFVMIMQQLKSLEYCVFVLIYELVLRRLRRTLSQLQEEFQDCEQDMLQALCVALK  
RNQLLLGRIRWLEGDVGSYFTPTMLLLFLYNGLTILHVMVNWAYINKFLYDSCCQYGPEY  
CLFVLLVYELILRTRHVLEQLKDDLEDFDCGARIQELCVTLKQNQLLIGRIWRLVDEIG  
AYFRWSMTLLFLYNGLTILHVNVWAIIRSIDPNDCCQLMSFHFSLNMEANRSRLAAAR  
PYIQIYSIFGLTPPIQFFTRTLHKRRRGIVILGYACYLISISLMVIYECYANIVALQKD

10081816.033002

IHKFHAEDSSKVMGNTQKVLVAMFVWNQLNILLNFRRLARIYDDIADLEIDLNNASSG  
FVGQRHWWRRFRRLALSVGLWIVLLVGLTPRFTLVALGPYLHWTNKVLTEIILIMLQLK  
CTEYCVFVLLIYELILRGRHILQQISVELEGNQSRDSVQELCVALKRNQLLAGRIWGLV  
NEVSLYFTLSLTLLFLYNELTILQIVNWALIKSVNPNECCQYTEDYLILKMGLREYSLO  
MEHLKLIFTGGLFDINLKFFGGVVKLKL

**Gr98A2** (SEQ ID NO: 18)

MEAKRSRLLTARPYLQVLSLFGLTPPAEFFTRTLRKRRRFCWMAGYSLYLIAILLMVF  
YEFHANIVSLHLEIYKFHVEDFSKVMGRTQKFLIVAIATCNQLNILLNYGRGLIYDEI  
ANLDLGIDKSSKNFCGKSHWWSFRLRLTSLIGLWMVIIIGVIPRLTLGRAGPFFHWVNQ  
VLTQIILIMLQLKGPEYCLFVLLVYELILRTRHVLEQLKDDLEDFDCGARIQELCVTLK  
QNQLLIGRIWRLVDEIGAYFRWSMTLLFLYNGLTILHVWNWAIIRSIDPNDCCQLSEE

**Gr2940.1** (SEQ ID NO: 19)

MFRPSGSGYRQKWTGLTLKGALYGSWILGVFPFAYDSWTRTLRRSKWLIAYGFVLNAAF  
ILLVVTNDTESETPLRMEVFHRNALAEQINGIHDIQSLSMVSIMLLRSFWKSGDIERTL  
NELEDLQHRYFRNYSLEECISFDRFVLYKGFVSVLELVSMLVLELGMSPNYSQAQFFIGL  
GSLCLMLLAVLLGASHFHLAVVFVYRYVWIVNRELLKLVNKMAIGETVESERMDLLLYL  
YHRLLDLGQRLASIYDYQMVMVMVSFLIANVLGIYFFIIYSSISLNKSLDFKILVFVQAL  
VINMLDFWLNVEICELAERTGRQTSTILKLFNDIENIDEKLEKLSVSFTSQHYCETDFAL  
FCSHRRLRFHHCGLFYVNYEMGFRMAITSFLYLLFLIQFDYWNL

**Gr2940.2** (SEQ ID NO: 20)

MVKQAEDREHGIMLDVFQRNALLYQISSLMGVGVVSICTVHLRTLWRSKHLEEIYNGL  
MLLEAKYFCSNAVECPAFDGYVIQKGVVIVGLLAPWMVHFGMPDSKLPVLNVLVSMV  
KLGTLALLALHYHLGVVVIYRFVWLINRELLSLVCSLRGNHKGSSSRVRFLKLYNKLVN  
LYSKLADCYDCQTVLMMAIFLAANIIVCFYMIVYRISLSKMSFFVMLIMFPLAIANNFM  
DFWLSMKVCDLLQKTGRQTSMLKLFNDIENMDKDLEISISDFALYCSHRRFKFLHCGL  
FHVNREMGMFVSVLVLYLLYLQF

**Gr2940.3** (SEQ ID NO: 21)

MFASRDLQSRCLCWIILKATLYSSWFLGVFPYRFSRNGQLKRSRFLLFYGLIILNFFLL  
LKMVCSGGQKLGIPEAFARNSVLENTHTTGMLAVFSCVVIHFLNFWGSTRVQDLANEL  
LVLEYQQFASLNETKCPKFNSFVIQKWSVIGLLLSYLSIAYGLPGNNFSVEMVLINSL

10081816 033002

VQFSFNCNIMHYIIGVLLIYRYLWLINGQLLEMVTNLKLDSCVSDSSRIRKYLSLYRRL  
ELKGYMVATYEHMTLVLTGLASNFLAIYSWIVLDISMNINFIYLLIFPLFLLNVWN  
LWLSIAASDLAENAGKSTQTVLKLFLADLEVKDIELERSVSVNSNRYKQVNEFALLCGH  
CQFNHVCGLFTINYKMGFQMIITSFLYLIYMIQFD

**Gr2940.4** (SEQ ID NO: 22)

MINVVIGIINVLSALIVHFMNFWGSRKVGEICNELLILEYQDFEGLNGRNCPNFNCFV  
IQKCLTILGQLLSFFTLNFALPGLEFHICLVLLSCLMEFSLNINIMHYHVGVLIIYRY  
VWLINEQLKDLVSQKLNPEFDTSRIHQFSLYKRLLELNKRLVIAYEYQMTLFI  
IAQLSGNIVVIYFLIVYGLSMRTYSIFLVAFPNSLLINIWDFWLCIAACDLTEKAGDETAI  
ILKIFSDLEHRDDKLEKFRFQLCGLFSMNCRMGFKMIITTFLYLVYLQFDYMN\*

**Gr2940.5** (SEQ ID NO: 23)

MSQPKRIHRICKGLARFTIRATLYGSWVLGLFPFTFDSRKRLNRSKWLLAYGLVLNL  
TLLVLSMLPSTDDHNSVKVEVFQRNPLVKQVEELVEVISLITTLVTHLRFTSRSELV  
EILNELLVLDKNHFSKMLLSECHTFNRYVIEKGLVIIIEIGSSLVLYFGIPNSKIVVY  
EAVCIYIVQLEVLMMVHFLAVIYIYRYLWIINGQLLDMASRLRRGDSVDPDRIQLL  
LWLYSRLLDLNHRILTAYIDIQVTLFMATLFSVNIIVGHVLVICWINITRFSLLVIFLL  
FPQALIINFWDLWQGIACDLAESTGKKTSMILKLFNDMENMDQETERRVSEYMFQNL  
MYFKYFKHPLIFVAEFTLFCSHRRLKVCHLGLLDINYEMGFRMIITNILYVVFLVQFD  
YMN

Previously reported Gustatory Receptors which are family members:

a) Full-length clones

**Gr21D1** (SEQ ID NO: 24)

MGVMPIHRNPPEKNLPRTGYSWGSQVMWAIFIYSCQTTIVVLRLRERVKKFVTSPDK  
RFDEAIYNVIFISLLFTNLLPVASWRHGPQVAIFKNMWTNYQYKFFKTTGSPIVFPN  
LYPLTWSLCVFSWLLSIAINLSQYFLQPDFRLWYTFAYYPIIAMLNCFCSLWYINCNA  
FGTASRALSDALQTTIRGEKPAQKLTEYRHLWVDLSHMMQQLGRAYSNMYGMYCLVIF  
FTTIIATYGSISEIIDHGATYKEVGLFVIVFYCMGLLYIICNEAHYASRKVGLDFQTK  
LLNINLTAVDAATQKEVEMLLVAINKNPPIMNLDGYANINRELITTNISFMATYLVVL  
LQFKITEQRRIQQQA

2008-10-09 15:00:00

**Gr22B1** (SEQ ID NO: 25)

MFQPRRGFSCHLAWFMLQTTLYASWLLGLFPFTFDSRRKQLKRSRWLLLYGFLHSL  
AMCLAMSSHLASKQRRKYNAFERNPLLEKIYMQFQVTTFFTTISVLLLMNVWKSNTVR  
KIANELLTLEGQVKDLLTLKNCPNFNCFVIKKHVAAGQFVISIYFCLCQENSYPKI  
LKILCCLPSVGLQLIIMHFHTEIILVYRYVWLNETLEDSSHLSRSSRIHALASLYDR  
LLKLSELVVACNDLQLILMLIIYLGINTVQIFFLIVLGVSMNKRYIYLVASPQLIIN  
FWDFWLNIIVCDLAGKCGDQTSKVLKLFDTLEHDDEELERSLNEFAWLCTHRKFREFQ  
LCGLFSINHNMGFQMIITSFLYLVYLLQFDFMNL

**Gr23A1a** (SEQ ID NO: 26)

MKTLECLTRRFLEVIFSVLALVPLPPISQLGWLFSLAIRCCWIVYFIYLLDVAISF  
SWVAIENVGNAVGTMLFVGNSVLGFALLLESVLKQKTHSQLEDLRVQTELQQLQRLGM  
FGRSRHAAYLLPLIGVQFTCDLVRLATNFGETVSPVFCISLPLMWLLRYRYVQLVQH  
VMDLNQRSIHLRRSLLSMASGNDLWQPYGVQECLQLQTLRTTYERIFECYETFSDCY  
GWGMLGLHLLTSFQFVTNAYWMIMGIYDGGNVRSLIFNGATGIDFGTPIATLFWHGD  
SGAENGRQIGCLISKLVKPPQGSKLYNDLVSEFSLQTLHQRFVVTAKDFFSLNLHLLS  
SMFAAVVTYLVILIQFMFAERSSTRGSG

**Gr23A1b** (SEQ ID NO: 27)

MFPPTRVQASSRVVLKIFHFILVAFSLRSRRLSRLVLWLQFLGWLTWFISMWTQSVIY  
AQTIDCTLDCSLRHILTFQTVSHAFIVVTSFLDGFRIKQDQLDEPIAFEDSDPWLA  
TVLAMLVPTLGVEYLVCSNAPEYAFRIRIYHLKTLPSFLALQVQIISFILEVMKVNIR  
VRQTKLQLLILARELSRWPPQRKQKPPQSDQQAHRVKDLKRRYNDLHYLFVRINGYFG  
GSLLTIIIVHFAIFVSNSYWLFDIRTPWRIYAILNLGFI FNVALQMAAACWHCQQ  
SYNLGRQIGCLISKLVKPPQGSKLYNDLVSEFSLQTLHQRFVVTAKDFFSLNLHLLSS  
FAAVVTYLVILIQFMFAERSSTRGSG

**Gr32D1** (SEQ ID NO: 28)

MPIYEQVSDYEVGPPTKTNEFYSSFFVRGVVHALTIFNVYSLFTPISAQLFFSYRETDN  
VNQWIELLLCILTYTLTVFVCAHNTTSMRLIMNEILQLDEEVRROFGANLSQNFGLV  
KFLVGITACQAYIIIVLKIYAVQGEITPTSYILLAFYGIQNGLTATYIVFASALLRIV  
IRFHFINQLNGYTYGQQHRRKEGGARARRQRGDVNPVNPALMEHFPEDSLFIYRMH  
NKLLRIYKGINDCCNLILVSFLGYSFYTVTTNVCYNLFVQITGKGMVSPNIIQWCFWL

20220929T001

CLHVSLLALLSRSCGLTTTEVSNYIGDKISIFMSVFISRPMPHPKFLQGCMPSSRRSIR  
ISGFHYQIDKFLTKSIKQEVQFTAYGFFAIDNSTLFKIFSAVTTYLVILIQFKQLEDS  
KVEDPVPEQT

**Gr39D1** (SEQ ID NO: 29)

MLYSFHPYLKYFALLGLVPWSESCAQSKFVQKVYSAILIILNAVHFGISIIYFPQSAE  
LFLSLMVNVIVFVARIVCVTVIILQVMVHYDDYFRFCREM KYLGLRLQCELKIHVGR  
LKWQSYAKILALGIGFLVTVLPSIYVALSGSLLYFWSSLLSILIIIRMQFVLVLLNVE  
LLGHHVSLLGIRLQNVLECHLMGANCTLDGNANRLCSLEFLLALKQSHMQLHYLFTH  
FNDLFGWSILGTYYVLFSDSTVNIYWTQQVLVEVYKYLYATFSVFVPSFFNILVF  
CRCGEFCQRQSVLIGSYLRNLSCHPSIGRETSYKDLLMEFILQVEQNVLAINEGFM  
STDNSLLMSILAAKVTYLIVLMQFSSV

**Gr39D2a** (SEQ ID NO: 30)

MGTRNRKLLFFLHYQRYLGLTNLDFSKSLHIYWLHGTWSSTAIQIVVVGVFMAALLG  
ALAESLYMETKSQTGNTFDNAVILTTSVTQLLANLWLRSSQKSQVNLLQRLSQVVE  
LLQFEPYAVPQFRWLYRIWLLVCLIIYGAMVTHFGINWLTMMQISRVLTIGFVYRCV  
LANFQFTCYTGMVVILKLLQVQVKQLEHLVSTTTISMAGVAGCLRTHDEILLGQR  
ELIAVYGGVILFLFIYQVMQCILIFYISNLEGFHSSNDLVLIFCWLAPMLFYLILPL  
VVNDIHNQANKTAKMLTKVPRTGTGLDRMIEKFLKNLRQKPILTAYGFFALDKSTL  
FKLFTAIFTYMVILVQFKEMENSTKSINKF

**Gr39D2b** (SEQ ID NO: 31)

MDFQPGELCAYYRLCRYLGIFCIDYNPTKKKFRLRRSVLCYIVHFALQAYLVGCISV  
MVTYWRRCFKSELTTTGNHFDRLVMVIALGILVVQNAWLIWLQAPHLRIVRQIEFYR  
RNHLANVRLLLPKRLLWLIATNVVYMANFIKTCIFEWLTDASRLFVITSLGFPLRY  
LVTSFTMGTYFCMVHIVRLVLDWNQSQINAIIDESADLKMTSPNRLRLRVCMHHR  
LMLLCNDEISLVYGFIAWLSWMFASLDVTGVIYLTMVIQTKKSIVLKLITNVVWLSP  
TFMTCAASFMSNRVTIQANKTAKMLTKVPRTGTGLDRMIEKFLKNLRQKPILTAYG  
FFALDKSTLFLKFTAIFTYMVILVQFKEMENSTKSINKF

**Gr39D2c** (SEQ ID NO: 32)

MKRNAFEELRVQLRTLKWLGVLRFTIDFNKCLVRENASEERSAWLYLIGVVGITCSL  
IVYSTYFSPSHFIMGKHNTTGNCYALINIRSCSIVTMLIYTQLYIQRFRRFVALLQSIL

RFNQISGSHREEGRFAFYTHLSLLIICMLNYAGYWTAGVRLTTIPIYLLQYGFS  
 YLFLGQVVVLFACIQQILLISILKYNNQVVLKNIKSSKESREFYNNFCKYNQVIWLSY  
 TEINHCFGLLLLLVLTGLILLITPSGPFYLVSTIFEGRFRQNWQFSLMSFTAILWSLP  
 WIVLLVLAMGRNDVQKEANKTAKMLTKVPRTGTGLDRMIEKFLLKNLRQKPILTAYG  
 FFALDKSTLFLKLTAFITYMVILVQFKEMENSTKSINKF

**Gr39D2d** (SEQ ID NO: 33)

MSKVCRLDRIYLRLLHIMGMMCWHFSDHCQLVATSGSERYAVVYAGCILVSTTAGF  
 IFALLHPSRFHIAIYNQTGNFYEAIVFRSTCVVLFLVYVILYAWRHRYRDLVQHILR  
 LNRRCASSCTNQQLHNIILYGMLTILCFGNYLHGYTRAGLATLPLALCMLVYIFAF  
 LVLCLLLMFFVSLKQVMTAGLIHYNQQLCQGDLSGLRGRQQILKLCGGELNECFGL  
 LMLPIVALVLLMAPSGPFFLISTVLEGKFRPDECLIMLLTSSTWDTPWMIMLVMLR  
 TNGISEEANKTAKMLTKVPRTGTGLDRMIEKFLLKNLRQKPILTAYGFFALDKSTLF  
 KLFTAFITYMVILVQFKEMENSTKSINKF

**Gr43C1** (SEQ ID NO: 34)

MKSATSKVVTALDVSVVVMAIVSGVYCGFLSLNDTLELNDRLNKIDNTLNAYNNFRD  
 RWRALGMAAVSLLAISILVGLDVGTWMRIAQDMNIAQSDTELNVHWYIPFYSLYFILT  
 GLQVNIANTAYGLGRRFGRLNRMLSSSFLAENNATSAIKPQKVSTVKNVSVNRPAMPS  
 ALHASLTKLNGETLPSEAAGDKAAARSLILNVELLKLGYFPAKNKGLLLKSLADSHES  
 LGKCVHLLSNSFGIAVLFILVSCLLHLVATAYFLFLELLSKRDNGYLWVQMLWICFHF  
 LRLLMVVEPCHLAARESRTIQIVCEIERKVHEPILAEAVKKFWQQLLVVDADFSACG  
 LCRVNRTILTSFASAIATYLVILIQFQRTNG

**Gr47A1** (SEQ ID NO: 35)

MAFTSSQLCSLLTKFTALNGLNTYYFDTKTNAFRVSSKLKIYCAIHHALCVLALAHMS  
 YSTASNLRVSVTVLTIGGTMACCVKSCWEKAQGIRNLARGLVTEQKYFAGRPSGLLL  
 KCRYIYKITFGSITLLRIHLIQPIYMRLLPSQFYLVNGAYWLLYNMLLA AVLGFYFL  
 LWEMCRIQKLINDQMTLILARSGQRNRLKKMQHCLRLYSKLLLLCDQFNSQLGHVAIW  
 VLACKSWCQITFGYEIFQMVAAPKSIDLTMSMRVFVIFTYIFDAMNLF LGTDISELFS  
 TFRADSQRILRETSRLDRLLSMFALKLALHPKRVVLLNVFTFDRKLTLLAKSTLYT  
 ICCLQNDYNKLKA

**Gr58A1** (SEQ ID NO: 36)

MLLKFMYYIGIGCGLMPAPLKKGQFLLGYKQRWYLIYTACLHGGLLTVLPFTTFPHYMY  
DDSYMSSNPVLKWTFLTNITRIMAMFSGVLLMWFRKRILNLGENLILHCLKCKT  
LDNRSKKYSKLRKRVRNVLFQMLLVANLSILLGALILFRIHSVQRISKAMIVAH  
TQFIYVVFMMTGICVILLVLHWQSERLQIALKDLCFLNHEERNSTLSENKANRS  
LGKLAKLFLFAENQRLVREVFRTFDLPIALLLLKMFVTNVNLVYHGVQFGNDTIE  
TSSYTRIVGQWVVISHYWSAVLLMNVDVTRRSDLKMGDLLREFSHLELVKRDFH  
LQLELFSDDLCHPSTYKVCGLFIFNKQTSLAYFFYVLVQVLVLVQFDLKNKVEKR  
N

**Gr58A2** (SEQ ID NO: 37)

MLHPKLGRVMNVVYHVSFVFLMSTTLRIRSCRKCLRLEKVSRTYTIYSFFVGIFLFLN  
LYFMVPRIMEDGYMKYNIVLQWNFFVMLFLRAIAVVSCTGLWLKRHKIIQLYKYSLIY  
WKRFGHITRAIVDKKELLDLQESLARIMIRKIIILLYSAFLCSTVLQYQLLSVINPQIFL  
AFCARLTHFLHFLCVKMGGFVGLVLLNHQFLVIHLAINALHGRKARKKWKALRSVAAMH  
LKTLLRLARRIFDMFDIANATVFINMFMNTAINILYHAVQYSNSSIKSNGWGILFGNGLIV  
FNFWGTMALMEMLDVVTSCNNTGQQLRQLSDLPKVGPKMQRELDYFTMQLRQNRLVYK  
ICGIVELDKPACLSYIGSILSNVILMQFDLRRQRQPINDRQYLIHLMKNKTKV

**Gr58A3** (SEQ ID NO: 38)

MNQYFLLHTYFQVSRLIGLCNLHYDSSNHRFILNHVPTVYCVILNVVYLLVLPFALF  
VLTGNIYHCPDAGMFGVVYNVVALTKLLTMLFLMSSVWIQRRRLYKLGNDLMKMLHKF  
RFNLGNDCRNRCLCKGLLTSSRFVLLTQQLLTRDSVVNCESNSSLRQAMVPYQSAAIV  
YALIMILLMSYVDMTVYMVEVAGNWLLVNMTQGVREMVQDLEVLPERNGIPREMGLMQ  
ILAAWRKLWRRCRRLDALLKQFVDIFQWQVLFNLLTTYIFSI AVLFRLLWIYLEFDKNF  
HLWKGILYAIIFLTHHVEIVMQFSIFEINRCKWLGLLEDVGNLWDINYSGRQCIKSSG  
TILSRKLEFSLLYMNRKLQLNPKVRRLHIVGLFDISNLTVHNMTRSIITNVLVLCQI  
AYKKYG

**Gr59D1** (SEQ ID NO: 39)

MADLLKLCLRIAYAYGRLTGVINFKIDLKTGQALVTRGATLISVSTHLLIFALLLYQT  
MRKSVNVNMWKYANSLHEYVFLVIAGFRVVCVFLELVSRWSQRRTFVRLFNSFRRLYQ  
RNPDI IQYCRRSIVSKFFCVTMTETLHIIVTLAMMRNLSIALALRIWAVLSLTAIN  
VIITQYYVATACVRGRYALLNKDLQAI VTESQSLVPNGGGVFVTKCCYLADRLERIAK

SQSDLQELVENLSTAYEGEVVCLVITYYLNMLGTSYLLFSISKYGNFGNNLLVIITLC  
GIVYFVYFVYVDCWINAFNVFYLLDAHDKMVKLLNKRTLFPGLDHRLEMVFENFALNL  
VRNPLKLHMYGLFEFGRGTSFAVFNSLLTHSLLLIQYDVQNF

**Gr59D2** (SEQ ID NO: 40)

MVDLVKTILLIAYWYGLAVGVSNFEVDWLTGEAIATRRTTIYAAVHNASLITLLILFN  
LGNNSLKSEFISARYLHEYFFMLMTAVRISAVLLSLITRWYQRSRFIRIWNQILALVR  
DRPQVVRGRWYRRSIIILKFVFCVLSDSLHTISDVSAQRKRITADLIVKLSLLATLTTI  
FNMIVCQYYLAMVQVIGLYKILLQDLRCLVRQAECICSIRNRRGGVYSIQCCSLADQL  
DLIAERHYFLKDRLEDMSDLFQIQSLSMSLVYFFSTMGSIYFSVCSILYSSTGFGSTY  
WGLLLIVLSTASFYMDNWLSVNIGFHIRDQQDELFRVLADRTLPHYRELDNRLEAAFEN  
FQLQLASNRHEFYVMGLFKMERGRLIAMLSSVITHMTMLVQWEIQN

**Gr59E1** (SEQ ID NO: 41)

MRSSATKGAKLKNSPRERLSSFNPOYAERYKELYRTLFWLLLIISVLANTAPITILPGC  
PNRFYRLVHLSWMILWYGLFVLGSYWEFVLVTTQRVSLDRYLNAIESAIYVVHIFSIM  
LLTWQCRNWAPKLMTNIVTSDLNRAYTIDCNRTKRFIRLQLFLVGIFACLAIFFNIWT  
HKFVVYRSILSINSYVMPNIISSISFAQYYLLLQGIAWRQRRLTEGLERELTHLHSPR  
ISEVQKIRMHHANLIDFTKAVNRTFQYSILLFLVGCFLNFNVLFLVYQGIENPSMAD  
FTKWVCMLLWLAMHVGKVCILHFNQSIQNEHSTCLTLLSRVSYARKDIQDTITHFII  
QMRTNVRQHVVCGVINLDLFLTLTLLVASADFFIFLLQYDVTYEALSKSVQGNVTRY

**Gr59E2** (SEQ ID NO: 42)

MDSSYWENLLLTINRFLGVYPSGRVGVLRWLHTLWSLFLLMYIWTGSIVKCLEFTVEI  
PTIEKLLYLMEFFPGNMATIAILVYYAVLNRPLAHGAELQIERIITGLKGKAKRLVYKR  
HGQRTLHLMATTLVFHGLCVLVDVVNYDFEFWTTWSSNSVYNLPGLMMSLGVLYQAQP  
VHFLWLVMQMRMCKELKLLQRPQGSTKLDACYESAFAVLVDAGGGSALMIEEMRY  
TCNLIEQVHSQFLLRFGLYLVNLLNSLVSI CVELYLI FNF FETPLWEESVLLVYRLL  
WLAMHGGRIFILSVNEQILEQKCNLCQLLNELEVCSSRLQRTINRFLQLQRSIDQP  
LEACGIVTLDTRSLGGFIGVLMAIVIFLIQIGLGNKSLMGVALNRSNWVYV

**Gr68D1** (SEQ ID NO: 43)

MKIYQDIYPISKPSQIFAILPFYSGDVDDGFRFGGLGRWYGRLVALIILIGSLTLGED  
VLFASKEYRLVASAQGDTEEINRTIETLLCII SYTMVVLSSVQNASRHFRTLHDIKI

2020.09.16 10:08:19



DEYLLANGFRETYSCRNLTLILVTSAGGVLAVAFYYIHYRSGIGAKRQIILLIYFLO  
 LLYSTLLALYLRTLMMNLAQRIGFLNQKLDTFNLQDCGHMENWRELSNLIIEVLCKFRY  
 ITENINCVAGVSLLFYFGFSFYTVTNQSYLAFATLTAGSLSSKTEVADTIGLSCIWVL  
 AETITMIVICSACDGLASEVNGTAQILARIYGKSKQFQNLIDKFLTKSIKQDLQFTAY  
 GFFSIDNSTLFKIFSAVTTYLVILIQFKQLED SKNLSRSYQLVM

**Gr77E1** (SEQ ID NO:44)

MPRWLQLPGMSALGILYSLTRVFGLMATANWSPRGIKVRQSLYLRIHGCVMILIFVGC  
 FSPFAFWCIFORMAFRLQRNILLMIGFNRYVLLLVCAMTLWIHCFKQAEIIGCLNRL  
 LKCRRLRLRLMHTRKLKDSMDCLATKGHLLLEVVLSSYLLSMAQPIQILKDDPEVRR  
 NFMYACSLVFSVCQAILQLSLGMYTMAILFLGHLVRHSNLLLAKILADAEHIFESSQ  
 KAGFWPNRQELYKGQKWLALWRLHVVHQLLKLHRSICSLCAVQAVCFLGFVPLE  
 CTIHLFFTYFMKYSKFILRKYGRSFPLNYFAIAFLVGLFTNLLLVLPTYYSERRFNC  
 TREIIKGGGLAFPSRITVKQLRHTMHFYGLYLNVEHVFAVSACGLFKLNNAILFCIV  
 GAILEYLMILIQFDKVLN

b) Previously reported partial Gustatory Receptor sequences. Predicted proteins have been extended as disclosed in the subject application; extended sequence information is indicated in **bold font**.

**Gr28A1** (SEQ ID NO: 45)

CQLNGYRTEHAGGNYLLASDFDRRLKVFLQWKTSDSAESASGRLGSQYTFVGHKKKQ  
 TGLTIKLAENGFCWVLLRLRYFSVLIVKYKIP

**Gr57B1** (SEQ ID NO: 46)

MAVLYFFREPETVFDCAAFICILQFLMGCNGFGIRRSTFRISWASRIYSMSVAIAAFC  
 CLFGSLSVLLAEEDIRERLAKADNLVLSISALELLMSTLVFGVTVISLQVFARRHLGI  
 YQRLAALDARLMSDFGANLNYRKMLRKNIAVLGIVTTIYLMAINSAAVQVASGHRALF  
 LLFALCYTIVTGGPHFTGYVHMTLAEMLGIRFRLQQLLQPEFLNWRFPQLHVQELRI  
 RQVVSMIQELHYLIQEINRVYALSLWAAMAHDLMSTSELYILFGQSVGIGQQNEEEN  
 GSCYRMLGYLALVMIPPLYKLLIAPFYCDRTIYEARRCLRLVEKLDDWFPQ**KSSLRPL**  
**VESLMSWRIQAKIQFTSGLDVVLSRKVIGLFTSILVNYLLILIQFAMTQKMGEQIEQQ**  
**KIALQEWIGF**

**Gr65C1** (SEQ ID NO: 47)

MRVHQRSAAV I I Q M G H P P F M S L K G G K S G F G S I V W P S A M R E V N L L N R F T R Q F L F L I V L  
V T Q I C G V A T F V Y N S K A Q C F R Q S G F L R F Y S S L V L I F L A L F L I V T T S K M F H N L Q A V W P Y  
V V G S V I I L V V R I H G L L E S A E I V E L L N Q M L R I M R Q V N L M A R H P N L F R L K H L L L L L L A L  
Q N L L R S L N T I V G I S N H S A E A Y D S F L N S V I L L I I L A V L L S F L L Q I T I N I C L F V V L I A T  
Y S E L H H C T R R I S N D M D K L R L H S V H E S G Q F M V L V K Q L Q G I T E K L I R L R Q N V F H I T V R I  
I R H F R F H W L C A I I Y G L L P F F S L T A K D Q N G F N F L I I S A L N I I F Q W T I F A I L S R E S

**Gr93F1** (SEQ ID NO: 48)

M T G K R A E S W S R L L L L W L Y R C A R G L L V L S S S L D R D K L Q L K A T K Q G S R N R F L H I L W R C I  
V V M I Y A G L W P M L T S A V I G K R L E S Y A D V L A L A Q S M S V S I L A V I S F V I Q A R G E N Q F R E V  
L N R Y L A L Y Q R I C L T T R L R H L F P T K F V V F L L K L F F T L C G C F H E I I P L F E N S H F D D I S  
Q M V G T G F G I Y M W L G T L C V L D A C F L G F L V S G I L Y E H M A N N I I A M L K R M E P I E S Q D E R Y  
R M T K Y R R M Q L L C D F A D E L D E C A A I Y S E L Y H V T N S F R R I L Q W Q I L F Y I Y L N F I N I C L M  
L Y Q Y I L H F L N D D E V V F V S I V M A F V K L A N L V L L M M C A D Y T V R Q S E V P K K L P L D I V C S D  
M D E R W D K S V S L L L F E T F L G Q L Q T Q R L E I K V L G F F H L N N E F I L L I L S A I I S Y L F I L I Q  
F G I T G G F E A S E D I K N F A D

**Gr93F2** (SEQ ID NO: 49)

M Q F W F G E E L I N L V N R F L Q L F R R M Q S L T N S P K N R F G D R A E F L L M F S K V F S L L F V F M A F  
R L M L S P W F L L T L V C D L Y T S V G T G M I T H L C F V G Y L S I G V L Y R D L N N Y V D C Q L R A Q L R S  
L N G E N N S F R N N P Q P T R Q A I S N L D K C L Y L Y D E I H Q V S R S F Q Q L F D L P L F L S L A Q S L L A  
M S M V S Y H A I L R R Q Y S F N L W G L V I K L L I D V V L L T M S V H S A V N G S R L I R R L S F E N F Y V T  
D S Q S Y H Q K V S P G A I I L R I K Y N T F P I L Q L E L F L G R L Q H Q E L R V F P L G L F E V S N E L T L F  
F L S A M V T Y L V F L V Q

**Gr93F3** (SEQ ID NO: 50)

M I E R L K K V S L P A L S A F I L F C S C H Y G R I L G V I C F D I G Q R T S D D S L V V R N R H Q F K W F C L  
S C R L I S V T A V C C F C A P Y V A D I E D P Y E R L L Q C F R L S A S L I C G I C I I V V Q V C Y E K E L L R  
M I I S F L R L F R R V R R L S S L K R I G F G G K R E F F L L L F K F I C L V Y E L Y S E I C Q L W H L P D S L  
S L F A T L C E I F L E I G S L M I I H I G F V G Y L S V A A L Y S E V N S F A R I E L R R Q L R S L E R P V G G  
P V G R K Q L R I V E Y R V D E C I S V Y D E I E R V G R T F H R L L E L P V L I I L L G K I F A T T I L S Y E V  
I I R P E L Y A R K I G M W G L V V K S F A D V I L L T L A V H E A V S S R M M R R L S L E N F P I T D H K A W

HMKVSDLMVFLIKCIIFFSRLQWEMFLSRLNFFFEFRVRPLGLFEVSNEVILLFLSSMI  
TYFTYVVQ

Gr93F4 (SEQ ID NO: 51)

MSFYARFLSLVCFRLRKQKDNNVWLEEIWSNRSRWKWISVTLRIVPLCIYAFTYAEW  
ISNRMLITEKFLHSCSLVVSIPCYLSIIHLKICHGPEVTKLVNQYLHIFRLGTLDIR  
RRSQFGGRELFLILSVCCQIHEYVFILVIASRLCGFQHIIWVVSXYTYVFIICNSI  
MCFGFIWHLISLGVLYAELNDNLRFESGFQTAFLRKQQRIRVQKSMALFKEISSVVT  
LQDIFNVHLFLSALLTLLQVLVVWYKMIIDLGFSDFRIWSFSLKNLIQTLLPVLAIQ  
EAANQFKQTRERALDIFLVGKSKHWMKSVSKLINQGILQLIGLFNVSNELFLIIVSA  
MFCYLVFVTQCVIVYRRRYVI

Gr94E1 (SEQ ID NO: 52)

MDFTSDYAHRRMVKFLTIIILIGFMTVFGLLANRYRAGRERRFRFSKANLAFASLWAIA  
FSLVYGRQIYKEYQEGQINLKDATTLYSYMNITVAVINYVSQMIISDHVAKVLSKVPF  
FDTLKEFRLDSRSLYISIVLALVKTVAFPLTIEVAFILQQRQHPMSLIWTLYRLFP  
LIISNFLNNCYFGAMVVVKEILYALNRRLEAQLQEVNLLQRKDQLKLYTKYYRMQRFC  
ALADELDQLAYRYRLIYVHSGKYLTPMSLSMILSLICHLLGITVGFYSLYYAIADTLI  
MGKPYDGLGSLINLVFLSISLAEITLLTHLCNHLVATRRSAVILQEMNLQHADSRYR  
QAVHGFTLLVTVTKYQIKPLGLYELDMRLISNVFSAVASFLLILVQADLSQRFKMQ

Gr97D1 (SEQ ID NO: 53)

MRFLRRQTRRLRSIWQRSIPVRRFRGKLHTQLVTICLYATVFLNILYGVYLGFRFSFR  
KKFVFSKGLTIYSLFVATFFALFYIWNINYEISTGQINLRDTIGIYCYMNCVCLFNY  
VTQWEKTLQIIRFQNSVPLFKVLDSDISAMIVWRAFIYGLLKIVFCPLITYITLILY  
HRRSISESQWTSVTTTKTMLPLIVSNQINNCFGGVLANLIFAANRKLHGIVKEAN  
MLQSPVQMNHLKPYRMRRFCELADLLDELARKYGFTASRSKNYLRFTDWSMVLSMLM  
NLLGITMGCYNQYLAIADHYINEEPFDLFLAIVLVVFLAVPFLELVMVARISNQTIVE  
VIVI

Gr98B1 (SEQ ID NO: 54)

IERFVCAQLVHEAYKQFASNGFRFLDALGCYEHSALGRARPLSRRGYAIKVSDHPATP  
PHYHMPKPKPPSHLAVQHATLTSGLRQLSFSCVNCNCSRCCWSLPMHFRYIFNASLC  
NCQRQ\*GY\*TLSCRRHCTATKNISFSFCHISFVFLKYDPKNPQLR

GrLU1=Gr36B1 (SEQ ID NO: 55)

MFDWVG LLLKVLYYYGQIIIGLINFEIDWQGRVVA AQRGILFAIAINV LICMV LLLQI  
SKKFNLDVYFGRANQLHQYVVIIVMVSLRMASLNRWRQRAQLMRLVECVLR LFLKKPHV  
KQMSRWAILVKFSVGVSNFLQMAISMESLDRLGFNEFVGMASDFWMSAIINMAISQH  
YLVILFVRAYYHLLKTEVRQAIHESQMLSEIYPRRAAFMTKCCYLADRIDNIAKLQNO  
LQSIVTQLNQVFGIQGIMVYGGYYIFSVATTYITYSLAINGIEELHLSVRAAALVFSW  
FLFYTSAILNLFVMLKLFDDHKEMERILEERTLFTSALDVRLEQSVSFYPTITELKY  
RDLVLSQFESIQLQLIRNPLKIEVLDIFTITRSSSAAMIGSIITNSIFLIQYDMEYF

GrLU2=Gr28A3 (SEQ ID NO: 56)

MWLLRRSVGKSGNRPHDVYTCYRLTIFMALCLGIVPYYVSISSEGRGKLTSSYIGYIN  
IIIRMAIYMVNSFYGAVNRDTLMSNFFLTDISNVIDALQKINGMLGIFAILLISLLNR  
KELLKLLATFDRLETEAFPRVLKNLAHQWDTRSLKAVNQKQ RSLQCLDSFSMYTIVTK  
DPAEIIQESMEIHHLICEAAATANKYFTYQLLTIISIAFLIIVFDAYYVLETLGKSK  
RESKFKTVEFVTF FSCQMILYLIAIISIVEGSNRAIKKSEKTGGIVHSLLNKTKSAEV  
KEKLQQFSMQLMHLKINF TAAGLFNIDRTLYFTISGALTTYLIILLQFTSNPNNGYG  
NGSSCCETFNNMTNHTL

GrLU3=Gr64A1 (SEQ ID NO: 57)

MKGPNNLNRKTPSKDNGVKQVESLARPETPPPKFVEDSNLEFNV LASEKL PNYTNLDL  
FHRAVFPPMFLAQCVAIMPLVGIRESNPRVRVFAYKSIPMFVTLIFMIATSILFLSMF  
THLLKIGITAKNFVGLVFFGCVLSAYVVFIRLAKKWP AVVRIWTRTEIPFTKPPYEIP  
KRNL SRRVQLAALAIIGLSLGEHALYQVSAILS YTRRIQMCANITTVPSFN NYMQTNY  
DYVFQLLPYSPIIAVLILATCTFVWNYMDLFIMMISKGLSYRFEQITTRIRKLEHEEV  
CESVFIQIREHYVKMCELLEFVDSAMSS LILLSCVNNLYFVCYQLLN VFNKL RWPIN Y  
IYFWYSLLYLIGRTAFVFLTAADINEESKRGLGVLRRVSSRSWCVEVERLIFQMTTQT  
VALSGKKFYFLTRLLFGMAGTIVTYELVLLQFDEPNRRKGLQP

GrLU4 (SEQ ID NO: 58)

IYILSLYIFFQFISNVSLIVVLKLF RDI

2023-03-15 10:08:15

GrLU7=Gr5A1 (SEQ ID NO: 59)

MRQLKGRNRCNRAVRHLKVQGKMWLKNLKSGLAQIRESQVRGTRKNFLHDGSFHEAV  
APVLAVAQCFCLMPVCGISAPTYRGLSFNRRSWRFWYSSLYLCSTSVDLAFSIRRVA  
HSVLDVRSVEPIVFHVSILIASWQFLNLAQLWPGLMRHWAVERRLPGYTCCCLQAR  
PARRKLVAFLVLLVSLMEHLLSIISVYYYDFCPRRSDPVESYLLGASAQLFEVFPY  
SNWLAWLGKIQNVLLTFGWSYMDIFLMMLGMGLSEMLARLNRSLEQQVRQPMPEAYW  
TWSRTLYRSIVELIREVDDAVSGIMLISFGSNLYFICLQLLKSINTMPSSAHAVYFY  
FSLLELLSRSTAVLLFVSAINDQAREPLRLRLVPLKGYHPEVFRFAAELASDQVAL  
TGLKFFNVTRKLFLAMAGTVATYELVLIQFHEDKKTWDCSPFNLD

The family of receptors disclosed herein has a signature motif which comprises consecutive amino acids having the following sequence:

-G-L/F-F-X-X-X-X-X-X-X-X-X-X-X-X-X-T-Y-L-V/I-L-V/I/L-Q-F- (SEQ ID NO: 60),

where X is any amino acid, and / means or.

The invention provides an isolated nucleic acid encoding an insect gustatory receptor protein, wherein the receptor protein comprises seven transmembrane domains and a C-terminal domain, and the C-terminal domain comprises consecutive amino acids having the following sequence:

-G-L/F-F-X-X-X-X-X-X-X-X-X-X-X-X-X-T-Y-L-V/I-L-V/I/L-Q-F- (SEQ ID NO: 60),

where X is any amino acid, and / means or.

20220916T0800

The invention provides an isolated nucleic acid encoding an insect odorant receptor protein, wherein the receptor protein comprises seven transmembrane domains and a C-terminal domain, and the C-terminal domain comprises consecutive amino acids having the following sequence:

-G-L/F-F-X-X-X-X-X-X-X-X-X-X-X-X-T-Y-L-V/I-L-V/I/L-Q-F- (SEQ ID NO: 60),

where X is any amino acid, and / means or.

The invention provides an isolated nucleic acid molecule encoding an insect gustatory receptor protein, wherein the nucleic acid molecule encodes a protein selected from the group consisting of:

- (a) an insect gustatory receptor protein comprising consecutive amino acids having the sequence of any of the receptors disclosed herein;
- (b) an insect gustatory receptor protein which shares from 7-50% amino acid identity with any one of the proteins of (a), and comprises seven transmembrane domains and a C-terminal domain, wherein the C-terminal domain comprises consecutive amino acids having the following sequence:

-G-L/F-F-X-X-X-X-X-X-X-X-X-X-X-X-T-Y-L-V/I-L-V/I/L-Q-F- (SEQ ID NO: 60),

where X is any amino acid, and / means or.

The invention provides an isolated nucleic acid molecule encoding an insect odorant receptor protein, wherein the nucleic acid molecule encodes a protein selected from the group consisting of:

- (a) an insect odorant receptor protein comprising consecutive amino acids having the sequence of any of the receptors disclosed herein;
- (b) an insect odorant receptor protein which shares from 7-50% amino acid identity with any one of the proteins of (a), and comprises seven transmembrane domains and a C-terminal domain, wherein the C-terminal domain comprises consecutive amino acids having the following sequence:

-G-L/F-F-X-X-X-X-X-X-X-X-X-X-X-X-X-X-T-Y-L-V/I-L-V/I/L-Q-F- (SEQ ID NO: 60),

where X is any amino acid, and / means or.

The invention provides an isolated nucleic acid encoding an insect gustatory receptor protein, wherein the nucleic acid molecule encodes a protein selected from the group consisting of:

- (a) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr2B1 in SEQ ID NO: 1,
- (b) an insect receptor protein comprising consecutive amino acids having a sequence

10081815-02202

identical to that set forth for Gr8D1 in SEQ ID  
NO: 2,

(c) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr10B1 in SEQ ID  
NO: 3,

(d) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr10B2 in SEQ ID  
NO: 4,

(e) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr28A2 in SEQ ID  
NO: 5,

(f) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr28A4 in SEQ ID  
NO: 6,

(g) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr33C1 in SEQ ID  
NO: 7,

(h) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr36B2 in SEQ ID  
NO: 8,

20220909 9:43:00



- (i) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr36B3 in SEQ ID NO: 9,
- (j) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr59C1 in SEQ ID NO: 10,
- (k) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr61D1 in SEQ ID NO: 11,
- (l) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr63F1 in SEQ ID NO: 12,
- (m) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr64A2 in SEQ ID NO: 13,
- (n) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for GR64A3 in SEQ ID NO: 14,
- (o) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr66C1 in SEQ ID NO: 15,

- 10081916-03300
- (p) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr92D1 in SEQ ID NO: 16,
  - (q) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr98A1 in SEQ ID NO: 17,
  - (r) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr98A2 in SEQ ID NO: 18,
  - (s) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr2940.1 in SEQ ID NO: 19,
  - (t) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr2940.2 in SEQ ID NO: 20,
  - (u) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr2940.3 in SEQ ID NO: 21,
  - (v) an insect receptor protein comprising consecutive amino acids having a sequence

identical to that set forth for Gr2940.4 in SEQ ID NO: 22,

- (w) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr2940.5 in SEQ ID NO: 23,
- (x) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr57B1 in SEQ ID NO: 46,
- (y) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr93F1 in SEQ ID NO: 48,
- (z) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr93F2 in SEQ ID NO: 49,
- (aa) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr93F3 in SEQ ID NO: 50,
- (bb) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr93F4 in SEQ ID NO: 51,

10081846-022022

- (cc) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr94E1 in SEQ ID NO: 52,
- (dd) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr93D1 in SEQ ID NO: 53,
- (ee) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for GrLU1=Gr36B1 in SEQ ID NO: 55,
- (ff) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for GrLU2=Gr28A3 in SEQ ID NO: 56,
- (gg) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for GrLU3=Gr64A1 in SEQ ID NO: 57,
- (hh) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for GrLU7=Gr5A1 in SEQ ID NO: 59, and
- (ii) an insect gustatory receptor protein which shares from 7-50% amino acid identity with any one of the proteins of (a)-(hh), and comprises seven transmembrane domains and a C-terminal

domain, wherein the C-terminal domain comprises consecutive amino acids having the following sequence:

-G-L/F-F-X-X-X-X-X-X-X-X-X-X-X-X-X-T-Y-L-V/I-  
L-V/I/L-Q-F- (SEQ ID NO: 60),

where X is any amino acid, and / means or.

In one embodiment, the insect odorant receptor protein shares at least 20% amino acid identity with any one of the proteins described herein. In one embodiment, the insect odorant receptor protein shares at least 30% amino acid identity with any one of the proteins described herein. In one embodiment, the insect odorant receptor protein shares at least 40% amino acid identity with any one of the proteins described herein. In one embodiment, the insect odorant receptor protein shares at least 50% amino acid identity with any one of the proteins described herein. In one embodiment, the insect odorant receptor protein shares at least 60% amino acid identity with any one of the proteins described herein. In one embodiment, the insect odorant receptor protein shares at least 70% amino acid identity with any one of the proteins described herein. In one embodiment, the insect odorant receptor protein shares at least 80% amino acid identity with any one of the proteins described herein.

The invention provides an isolated nucleic acid molecule encoding an insect odorant receptor protein, wherein the nucleic acid molecule encodes a protein selected from the group consisting of:

- (a) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr2B1 in SEQ ID NO: 1,
- (b) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr8D1 in SEQ ID NO: 2,
- (c) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr10B1 in SEQ ID NO: 3,
- (d) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr10B2 in SEQ ID NO: 4,
- (e) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr28A2 in SEQ ID NO: 5,
- (f) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr28A4 in SEQ ID NO: 6,
- (g) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr33C1 in SEQ ID NO: 7,

- (h) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr36B2 in SEQ ID NO: 8,
- (i) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr36B3 in SEQ ID NO: 9,
- (j) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr59C1 in SEQ ID NO: 10,
- (k) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr61D1 in SEQ ID NO: 11,
- (l) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr63F1 in SEQ ID NO: 12,
- (m) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr64A2 in SEQ ID NO: 13,
- (n) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for GR64A3 in SEQ ID NO: 14,

- (o) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr66C1 in SEQ ID NO: 15,
- (p) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr92D1 in SEQ ID NO: 16,
- (q) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr98A1 in SEQ ID NO: 17,
- (r) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr98A2 in SEQ ID NO: 18,
- (s) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr2940.1 in SEQ ID NO: 19,
- (t) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr2940.2 in SEQ ID NO: 20,
- (u) an insect receptor protein comprising consecutive amino acids having a sequence



identical to that set forth for Gr2940.3 in SEQ  
ID NO: 21,

(v) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr2940.4 in SEQ  
ID NO: 22,

(w) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr2940.5 in SEQ  
ID NO: 23,

(x) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr57B1 in SEQ ID  
NO: 46,

(y) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr93F1 in SEQ ID  
NO: 48,

(z) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr93F2 in SEQ ID  
NO: 49,

(aa) an insect receptor protein comprising  
consecutive amino acids having a sequence  
identical to that set forth for Gr93F3 in SEQ ID  
NO: 50,

10081816-02200

- (bb) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr93F4 in SEQ ID NO: 51,
- (cc) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr94E1 in SEQ ID NO: 52,
- (dd) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for Gr93D1 in SEQ ID NO: 53,
- (ee) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for GrLU1=Gr36B1 in SEQ ID NO: 55,
- (ff) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for GrLU2=Gr28A3 in SEQ ID NO: 56,
- (gg) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for GrLU3=Gr64A1 in SEQ ID NO: 57,
- (hh) an insect receptor protein comprising consecutive amino acids having a sequence identical to that set forth for GrLU7=Gr5A1 in SEQ ID NO: 59, and

- (ii) an insect odorant receptor protein which shares from 7-50% amino acid identity with any one of the proteins of (a)-(hh), and comprises seven transmembrane domains and a C-terminal domain, wherein the C-terminal domain comprises consecutive amino acids having the following sequence:

-G-L/F-F-X-X-X-X-X-X-X-X-X-X-X-X-T-Y-L-V/I-L-V/I/L-Q-F- (SEQ ID NO: 60),

where X is any amino acid, and / means or.

In one embodiment, the insect gustatory receptor protein shares at least 20% amino acid identity with any one of the proteins described herein. In one embodiment, the insect gustatory receptor protein shares at least 30% amino acid identity with any one of the proteins described herein. In one embodiment, the insect gustatory receptor protein shares at least 40% amino acid identity with any one of the proteins described herein. In one embodiment, the insect gustatory receptor protein shares at least 50% amino acid identity with any one of the proteins described herein. In one embodiment, the insect gustatory receptor protein shares at least 60% amino acid identity with any one of the proteins described herein. In one embodiment, the insect gustatory receptor protein shares at least 70% amino acid identity with any one of the proteins described herein. In one embodiment, the insect gustatory receptor protein shares at least 80% amino acid identity with any one of the proteins described herein.

In one embodiment of any of the isolated nucleic acid molecules described herein, the insect gustatory or odorant receptor protein comprises seven transmembrane domains.

In different embodiments of any of the isolated nucleic acid molecules described herein, the nucleic acid is DNA or RNA. In different embodiments, the DNA is cDNA, genomic DNA, or synthetic DNA.

In one embodiment of any of the isolated nucleic acid molecules described herein, the nucleic acid molecule encodes a *Drosophila* receptor.

The nucleic acid molecules encoding an insect gustatory or odorant receptor include molecules coding for polypeptide analogs, fragments or derivatives of antigenic polypeptides which differ from naturally-occurring forms in terms of the identity or location of one or more amino acid residues (deletion analogs containing less than all of the residues specified for the protein, substitution analogs wherein one or more residues specified are replaced by other residues and addition analogs where in one or more amino acid residues is added to a terminal or medial portion of the polypeptides) and which share some or all properties of naturally-occurring forms.

These molecules include but not limited to: the incorporation of codons "preferred" for expression by selected non-mammalian hosts; the provision of sites for cleavage by restriction endonuclease enzymes; and the provision of additional initial, terminal or intermediate sequences that facilitate construction of readily expressed vectors. Accordingly, these changes may result

10034915-023303

in a modified insect receptor. It is the intent of this invention to include nucleic acid molecules which encode modified insect receptors. Also, to facilitate the expression of receptors in different host cells, it may be necessary to modify the molecule such that the expressed receptors may reach the surface of the host cells. The modified insect receptor should have biological activities similar to the unmodified insect gustatory or odorant receptor. The molecules may also be modified to increase the biological activity of the expressed receptor.

The invention provides a nucleic acid molecule comprising at least 12 nucleotides which specifically hybridizes with any of the isolated nucleic acid molecules described herein.

In one embodiment, the nucleic acid molecule hybridizes with a unique sequence within the sequence of any of the nucleic acid molecules described herein. In different embodiments, the nucleic acid is DNA, cDNA, genomic DNA, synthetic DNA, RNA, or synthetic RNA.

This invention provides a vector which comprises any of the isolated nucleic acid molecules described herein. In one embodiment, the vector is a plasmid.

In one embodiment of any of the vectors described herein, any of the isolated nucleic acid molecules described herein is operatively linked to a regulatory element.

Regulatory elements required for expression include promoter sequences to bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector includes a

promoter such as the lac promoter and for transcription initiation the Shine-Dalgarno sequence and the start codon AUG. Similarly, a eukaryotic expression vector includes a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the ribosome. Such vectors may be obtained commercially or assembled from the sequences described by methods well-known in the art, for example the methods described herein for constructing vectors in general.

The invention provides a host vector system for production of a polypeptide having the biological activity of an insect gustatory or odorant receptor, which comprises any of the vectors described herein and a suitable host. In different embodiments, the suitable host is a bacterial cell, a yeast cell, an insect cell, or an animal cell.

The host cell of the expression system described herein may be selected from the group consisting of the cells where the protein of interest is normally expressed, or foreign cells such as bacterial cells (such as *E. coli*), yeast cells, fungal cells, insect cells, nematode cells, plant or animal cells, where the protein of interest is not normally expressed. Suitable animal cells include, but are not limited to Vero cells, HeLa cells, Cos cells, CV1 cells and various primary mammalian cells.

The invention provides a method of producing a polypeptide having the biological activity of an insect gustatory or odorant receptor which comprising growing any of the host vector systems described herein under conditions permitting production of the polypeptide and recovering the polypeptide so produced.

The invention provides a purified insect gustatory or odorant receptor protein encoded by any of the isolated nucleic acid molecules described herein. This invention further provides a polypeptide encoded by any of the isolated nucleic acid molecules described herein.

The invention provides an antibody which specifically binds to an insect gustatory or odorant receptor protein encoded by any of the isolated nucleic acid molecules described herein. In one embodiment, the antibody is a monoclonal antibody. In another embodiment, the antibody is polyclonal.

The invention provides an antibody which competitively inhibits the binding of any of the antibodies described herein capable of specifically binding to an insect gustatory or odorant receptor. In one embodiment, the antibody is a monoclonal antibody. In another embodiment, the antibody is polyclonal.

Monoclonal antibody directed to an insect gustatory or odorant receptor may comprise, for example, a monoclonal antibody directed to an epitope of an insect gustatory or odorant receptor present on the surface of a cell. Amino acid sequences may be analyzed by methods well known to those skilled in the art to determine whether they produce hydrophobic or hydrophilic regions in the proteins which they build. In the case of cell membrane proteins, hydrophobic regions are well known to form the part of the protein that is inserted into the lipid bilayer which forms the cell membrane, while hydrophilic regions are located on the cell surface, in an aqueous environment.

Antibodies directed to an insect gustatory or odorant receptor may be serum-derived or monoclonal and are prepared using methods well known in the art. For example, monoclonal antibodies are prepared using hybridoma technology by fusing antibody producing B cells from immunized animals with myeloma cells and selecting the resulting hybridoma cell line producing the desired antibody. Cells such as NIH3T3 cells or 293 cells which express the receptor may be used as immunogens to raise such an antibody. Alternatively, synthetic peptides may be prepared using commercially available machines.

As a still further alternative, DNA, such as a cDNA or a fragment thereof, encoding the receptor or a portion of the receptor may be cloned and expressed. The expressed polypeptide may be recovered and used as an immunogen.

The resulting antibodies are useful to detect the presence of insect gustatory or odorant receptors or to inhibit the function of the receptor in living animals, in humans, or in biological tissues or fluids isolated from animals or humans.

This antibodies may also be useful for identifying or isolating other insect gustatory or odorant receptors. For example, antibodies against the *Drosophila* odorant receptor may be used to screen an cockroach expression library for a cockroach gustatory or odorant receptor. Such antibodies may be monoclonal or monospecific polyclonal antibody against a selected insect gustatory or odorant receptor. Different insect expression libraries are readily available and may be made using technologies well-known in the art.



One means of isolating a nucleic acid molecule which encodes an insect gustatory or odorant receptor is to probe a libraries with a natural or artificially designed probes, using methods well known in the art. The probes may be DNA, cDNA or RNA. The library may be cDNA or genomic DNA.

The invention provides a method of transforming a cell which comprises transfecting a host cell with any of the vectors described herein.

The invention provides a transformed cell produced by any of the methods described herein. In one embodiment, prior to being transfected with the vector the host cell does not express a gustatory or an odorant receptor protein. In one embodiment, prior to being transfected with the vector the host cell does not express a gustatory and an odorant receptor protein. In one embodiment, prior to being transfected with the vector the host cell does express a gustatory or odorant receptor protein.

This invention provides a method of identifying a compound which specifically binds to an insect gustatory receptor which comprises contacting any of the transformed cells described herein, or a membrane fraction from said cells, with the compound under conditions permitting binding of the compound to the gustatory receptor, detecting the presence of any such compound specifically bound to the receptor, and thereby identifying the compound as a compound which specifically binds to an insect gustatory receptor.

This invention provides a method of identifying a compound which specifically binds to an insect odorant receptor

which comprises contacting any of the transformed cells described herein, or a membrane fraction from said cells, with the compound under conditions permitting binding of the compound to the odorant receptor, detecting the presence of any such compound specifically bound to the receptor, and thereby identifying the compound as a compound which specifically binds to an insect odorant receptor.

This invention provides a method of identifying a compound which specifically binds to an insect gustatory receptor which comprises contacting any of the purified insect gustatory receptor proteins described herein with the compound under conditions permitting binding of the compound to the purified gustatory receptor protein, detecting the presence of any such compound specifically bound to the receptor, and thereby identifying the compound as a compound which specifically binds to an insect gustatory receptor.

This invention provides a method of identifying a compound which specifically binds to an insect odorant receptor which comprises contacting any of the purified insect odorant receptor proteins described herein with the compound under conditions permitting binding of the compound to the purified odorant receptor protein, detecting the presence of any such compound specifically bound to the receptor, and thereby identifying the compound as a compound which specifically binds to an insect odorant receptor.

In one embodiment, the purified insect gustatory or odorant receptor protein is embedded in a lipid bilayer. The purified receptor may be embedded in the liposomes

with proper orientation to carry out normal functions. Liposome technology is well-known in the art.

The invention provides a method of identifying a compound which activates an insect gustatory receptor which comprises contacting any of the transformed cells described herein, or a membrane fraction from said cells, with the compound under conditions permitting activation of the gustatory receptor, detecting activation of the receptor, and thereby identifying the compound as a compound which activates an insect gustatory receptor.

The invention provides a method of identifying a compound which activates an insect odorant receptor which comprises contacting any of the transformed cells described herein, or a membrane fraction from said cells, with the compound under conditions permitting activation of the odorant receptor, detecting activation of the receptor, and thereby identifying the compound as a compound which activates an insect odorant receptor.

The invention provides a method of identifying a compound which activates an insect gustatory receptor which comprises contacting any of the purified insect gustatory receptor proteins described herein with the compound under conditions permitting activation of the gustatory receptor, detecting activation of the receptor, and thereby identify the compound as a compound which activates an insect gustatory receptor.

The invention provides a method of identifying a compound which activates an insect odorant receptor which comprises contacting any of the purified insect odorant receptor proteins described herein with the compound under

conditions permitting activation of the odorant receptor, detecting activation of the receptor, and thereby identify the compound as a compound which activates an insect odorant receptor.

In one embodiment, the purified insect gustatory or odorant receptor protein is embedded in a lipid bilayer. The purified receptor may be embedded in the liposomes with proper orientation to carry out normal functions. Liposome technology is well-known in the art.

The invention provides a method of identifying a compound which inhibits the activity of an insect gustatory receptor which comprises contacting any of the transformed cells described herein, or a membrane fraction from said cells, with the compound under conditions permitting inhibition of the activity of the gustatory receptor, detecting inhibition of the activity of the receptor, and thereby identifying the compound as a compound which inhibits the activity of an insect gustatory receptor.

The invention provides a method of identifying a compound which inhibits the activity of an insect odorant receptor which comprises contacting any of the transformed cells described herein, or a membrane fraction from said cells, with the compound under conditions permitting inhibition of the activity of the odorant receptor, detecting inhibition of the activity of the receptor, and thereby identifying the compound as a compound which inhibits the activity of an insect odorant receptor.

The invention provides a method of identifying a compound which inhibits the activity of an insect gustatory receptor which comprises contacting any of the purified

insect gustatory receptor proteins described herein with the compound under conditions permitting inhibition of the activity of the gustatory receptor, detecting inhibition of the activity of the receptor, and thereby identifying the compound as a compound which inhibits the activity of an insect gustatory receptor.

The invention provides a method of identifying a compound which inhibits the activity of an insect odorant receptor which comprises contacting any of the purified insect odorant receptor proteins described herein with the compound under conditions permitting inhibition of the activity of the odorant receptor, detecting inhibition of the activity of the receptor, and thereby identifying the compound as a compound which inhibits the activity of an insect odorant receptor.

In one embodiment, the purified insect gustatory or odorant receptor protein is embedded in a lipid bilayer. The purified receptor may be embedded in the liposomes with proper orientation to carry out normal functions. Liposome technology is well-known in the art.

In one embodiment of any of the methods described herein, the compound is not previously known.

The invention provides a compound identified by any of the methods described herein. In one embodiment, the compound is an alarm odorant ligand or a ligand associated with fertility. In one embodiment the compound interferes with chemosensory perception.

The invention provides a method of combating ingestion of crops by pest insects which comprises identifying a

compound by any of the methods described herein and spraying the crops with the compound.

The invention provides a use of a compound identified by any of the methods described herein for combating ingestion of crops by pest insects.

The invention provides a use of a compound identified by any of the methods described herein for combating pest nuisances and disease-carrying insects by interfering with chemosensory perception.

The invention provides a method of combating disease-carrying insects in an area which comprises identifying a compound by any of the methods described herein and spraying the area with the compound.

The invention provides a method of controlling a pest population in an area which comprises identifying a compound any of the methods described herein and spraying the area with the compound. In one embodiment, the compound is an alarm odorant ligand or a ligand associated with fertility. In one embodiment the compound interferes with chemosensory perception.

The invention provides a method of controlling a pest population which comprises identifying a compound by any of the methods described herein, wherein the compound interferes with an interaction between an odorant ligand and an odorant receptor which are associated with fertility.

The invention provides a composition which comprises a compound identified by any of the methods described herein and a carrier.

The invention provides a method of preparing a composition which comprises identifying a compound by any of the methods described herein and admixing a carrier. The invention provides a method of preparing a composition which comprises identifying a compound by any of the methods described herein, recovering the compound free from the receptor, and admixing a carrier. The invention provides a method of preparing a composition which comprises identifying a compound by any of the methods described herein, recovering the compound from the cells or membrane fraction or receptor protein, and admixing a carrier. Examples of carriers include, but are not limited to, phosphate buffered saline, physiological saline, water, and emulsions, such as oil/water emulsions.

The invention provides a use of a compound identified by any of the methods described herein for preparing a composition for controlling a pest population in an area by spraying the area with the compound. In one embodiment, the compound is an alarm odorant ligand or a ligand associated with fertility. In one embodiment the compound interferes with chemosensory perception.

The invention provides a use of a compound identified by any of the methods described herein for preparing a composition for controlling a pest population. In one embodiment, the compound interferes with an interaction between an odorant ligand and an odorant receptor which are associated with fertility. In one embodiment the compound interferes with chemosensory perception.





## Experimental Details

### Materials And Methods

#### Experimental Animals

*Drosophila* stocks were reared on standard cornmeal-agar-molasses medium at 25° C. Oregon R strains were used for in situ hybridization experiments, and yw or W1118 strains were used for transgene injections. P-element mediated germline transformations and all subsequent fly manipulations were performed using standard techniques (Rubin et al., 1985). In some cases, transgenic constructs were injected as mixtures of two constructs, and progeny of individual transformants were analyzed by polymerase chain reaction (PCR) to determine their genotype. All analyses were performed on two to five independent transgenic lines for each construct.

#### Identification of additional GR genes

A search for novel seven transmembrane domain receptors was performed among 5660 predicted *Drosophila* proteins of 'unknown function' (Adams et al., 2000) using a transmembrane prediction program (TopPred) (von Heijne, 1992). 310 *Drosophila* genes were selected for in situ hybridization analysis, 20 of which were novel members of the GR gene family previously described (Clyne et al., 2000). Additional members of the GR gene family were identified using BLAST (Altschul et al., 1990) and hidden Markov model (Eddy, 1998) searches of *Drosophila* genome databases with existing GR members as templates. GRs were grouped into subfamilies by BLASTP comparisons (Altschul, et al., 1998) with an e value cutoff of  $10^{-5}$ . Sequence relationships between the GR gene family and the DOR genes were analyzed with HMMs (Eddy, 1998), CLUSTAL alignments

and neighbor joining trees (Saitou and Nei, 1987; Higgins and Sharp, 1988), and NxN BLASTP (Rubin et al., 2000) comparisons.

Five GR genes were isolated by PCR from proboscis cDNA using primers corresponding to the extent of the predicted coding region. Proboscis cDNA was obtained from one thousand microdissected probosces, using Dynal mRNA Direct (610.11) and Perkin-Elmer GeneAmp (N808-0017) kits. PCR products were cloned into pGEM-T (Promega) and sequenced in their entirety, using ABI 310 or 377 sequencing systems. An antennal cDNA library (kindly provided by Dr. Leslie Vosshall) was screened ( $3 \times 10^6$  inserts) with PCR probes for *Gr63F1*, *Gr10B1*, and *Gr21D1*, and 6 independent cDNAs of *Gr63F1* were isolated and sequenced. Sequences of *Gr43C1*, *Gr47A1*, *Gr58A3*, and *Gr59E1* matched the previously reported sequences (Clyne et al., 2000), and sequences of *Gr10B1* and *Gr63F1* are included in the list above.

#### ***In situ* hybridization**

RNA *in situ* hybridization was performed as previously described (Vosshall et al., 1999). Riboprobes for the 56 GR genes were generated from PCR products corresponding to predicted exons and ranged from 300-800 bp in length. Newly eclosed flies were used for *in situ* hybridization experiments because hybridization signals were found to be more robust at this stage.

#### **Construction of GR transgenes**

Generation of 15 GR promoter-Gal4 transgenes was performed as previously described (Vosshall et al., 2000). Briefly, sequences immediately adjacent to the predicted ATG initiation codon and a variable distance upstream were isolated by long range PCR with genomic DNA as template,

and upstream elements were cloned into a modified CaSpeR-AUG-Gal4 vector (Vosshall et al., 2000). Regulatory element lengths for each of the GR transgenes are as follows: *Gr2B1*, 2.240 kB; *G21D1*, 9.323 kB; *Gr22B1*, 8.249 kB; *Gr28A3*, 4.245 kB; *Gr32D1*, 3.776 kB; *Gr47A1*, 7.321 kB; *Gr66C1*, 3.153 kB and *Gr5A1*, 5.156 kB; *Gr10B1*, 0.656 kB; *Gr33C1*, 3.315 kB; *Gr39D2A*, 8.227 kB; *Gr59E2*, 2.586 kB; *Gr77E1*, 9.502 kB; *Gr93F1*, 9.368 kB; *Gr98A1*, 1.086 kB. The first 7 transgenes drive reporter expression in chemosensory tissues; the remaining 8 transgenes were not detectably expressed in adults or larvae.

#### **Visualization of lacZ, GFP, and nSyb-GFP reporters**

*GR promoter-Gal4* lines were crossed to *UAS-LacZ* stocks, and whole mount heads of progeny were examined for B-galactosidase activity, following existing staining procedures (Wang et al., 1998). To enhance visualization of sensilla in the proboscis labellum, probosces were bisected and pseudotracheae were removed by microdissection. Images were recorded using a Nikon SPOT-RT digital microscope system equipped with differential interference contrast.

Progeny resulting from crosses of *GR promoter-Gal4* to *UAS-GFP* were examined for GFP expression by direct fluorescence microscopy. Adult organs and live larvae were mounted in glycerol using small coverslips as spacers and GFP fluorescence was recorded with a BioRad 1024 confocal microscope.

To visualize axonal projections of GR-bearing neurons, *GR promoter -Gal4* flies were mated with *UAS-nSyb-GFP*, and brains of F1 progeny were examined by fluorescent immunohistochemistry. Larval brains were dissected and

antibody staining was carried out as described in (Vosshall et al., 2000). Expression of nSyb-GFP was visualized with a rabbit anti-GFP antibody (Molecular Probes) and a goat anti-rabbit secondary antibody coupled to Alexa Fluor 488 (Molecular Probes). The nc82 monoclonal antibody (Laissue et al., 1999) was used to label brain neuropil and was visualized with goat anti-mouse IgG coupled to CY3 (Jackson ImmunoResearch). Cell nuclei were counterstained with TOTO-3 (Molecular Probes). Images were analyzed with a BioRad 1024 confocal microscope.

## RESULTS

### A Large Family of Candidate Chemoreceptors

A novel family of putative seven transmembrane domain proteins was recently identified in searches of the *Drosophila* genome (Clyne et al., 2000). Analysis of a database representing 60% of the *Drosophila* genome identified twenty-three full-length genes and 20 partial sequences. The expression of 19 genes was examined by RT-PCR analysis and revealed 18 transcripts in the proboscis labellum, suggesting that this novel gene family may encode the fly gustatory receptors (GRs). The expression of these genes was characterized by *in situ* hybridization and transgene experiments and observe expression in both gustatory and olfactory chemosensory neurons in both larvae and adult flies.

The gene family has been extended by analyzing the recently completed euchromatic genome sequence of *Drosophila* (Adams et al., 2000) using reiterative BLAST searches (Altschul et al., 1990), transmembrane domain prediction programs (von Heijne, 1992), and hidden Markov model (HMM) analyses (Eddy, 1998). These searches have

identified a total of 56 candidate GR genes in the *Drosophila* genome, including 23 GRs not previously described. As originally reported, these genes encode putative seven transmembrane domain proteins of about 480 amino acids (Clyne et al., 2000). The family as a whole is extremely divergent and reveals an overall sequence identity ranging from 7-70%. However, all genes share significant sequence similarity within a 33 amino signature motif in the putative seventh transmembrane domain in the C-terminus (Figure 1). Analysis of the sequence of the 56 genes reveals the existence of four discrete subfamilies (containing ten, six, four and three genes) whose members exhibit greater overall sequence identity ranging from 40-70%. Twenty-two of the GR genes reside as individual sequences distributed throughout each of the *Drosophila* chromosomes, whereas the remaining genes are linked in the genome in small tandem arrays of two to five genes.

The GR family shares little sequence similarity outside of the conserved C terminal signature in the putative seventh transmembrane domain and therefore searches of the genome database are unlikely to be exhaustive. Thus, this family of candidate gustatory receptors consists of a minimum of 56 genes. Moreover, this analysis would not detect alternatively spliced transcripts, a feature previously reported for some members of this gene family (Clyne et al., 2000). cDNAs or RT PCR products were identified from six genes; verification of the gene predictions therefore awaits the isolation and sequencing of additional cDNAs.

Interestingly, the 33 amino acid signature motif characteristic of the GR genes is present but somewhat diverged in 33 of the 70 members of the family of

*Drosophila* odorant receptor (DOR) genes. (Figure 1). The DOR genes, however, possess additional conserved motifs not present in the GR genes and define a distinct family (Clyne et al., 1999; Vosshall et al., 1999; Gao and Chess, 1999; Vosshall et al., 2000). These observations suggest that the putative gustatory and olfactory receptor gene families may have evolved from a common ancestral gene.

#### **GR Gene Expression in Olfactory and Gustatory Organs**

Insight into the specific problem of the function of these candidate receptor genes and the more general question as to how tastants are recognized and discriminated by the fly brain initially requires an analysis of the patterns of expression of the individual GR genes in chemosensory cells. *In situ* hybridization was performed on sagittal sections of the adult fly head with RNA probes obtained from all 56 family members. Six of the genes are expressed in discrete, topographically-restricted subpopulations of neurons within the proboscis (Figure 2A). Three of the genes revealed no hybridization to the proboscis but are expressed in spatially-defined sets of neurons within the third antennal segment, the major olfactory organ of the adult fly (Figure 2B). The remaining genes show no hybridization to adult head tissues.

Our analysis of the pattern of GR gene expression by *in situ* hybridization demonstrates that a small number of GR genes is transcribed in either the proboscis or the antenna, suggesting that this family encodes chemosensory receptors involved in smell as well as taste. However, expression of over 80% of the family members was not detected using these *in situ* hybridization conditions. The sequence of these GR genes does not reveal nonsense or

frameshift mutations that characterize pseudogenes. The inability to detect transcripts from the majority of the GR genes by *in situ* hybridization might result from low levels of expression of GR genes, expression in populations of chemosensory cells not amenable to analysis by *in situ* hybridization (e.g., leg, wing, or vulva), or expression at other developmental stages.

Lines of flies expressing GR promoter transgenes were therefore generated to visualize the expression in a wider range of cell types with higher sensitivity. Transgenes were constructed in which putative GR promoter sequences (0.5-9.5 kb of DNA immediately upstream of the translational start) were fused to the Gal4 coding sequence (Brand and Perrimon, 1993). Flies bearing GR transgenes were mated to transgenic flies that contain either B-galactosidase (*lacZ*) or green fluorescent protein (GFP) under the control of the Gal4-responsive promoter, UAS. GR promoter-Gal4 lines were constructed with upstream sequences from 15 chemoreceptor genes and transgene expression was detected for 7 lines (Table 1). Five of the genes that were expressed by transgene analyses were also detected by *in situ* hybridization.

#### A spatial map of GR expression in the proboscis

Expression of the GR transgenes in the proboscis was initially visualized using the *UAS-lacZ* reporter. The labellum of the proboscis is formed from the fusion of two labial palps, each containing 31-36 bilaterally symmetric chemosensory bristles arranged in four rows (Figure 3) (Arora et al., 1987; Ray et al., 1993). The sensilla of the first three columns contains four chemosensory neurons and a single mechanoreceptor cell whereas the sensilla in the most peripheral row are composed of only two

10081816.02200

chemosensory neurons and one mechanoreceptor (Nayak and Singh, 1983; Ray et al., 1993). Each labial palp therefore contains approximately 120 chemosensory neurons.

The *GR promoter-Gal4* lines were crossed to *UAS-lacZ* flies and the progeny were examined for lacZ expression by staining of whole mount preparations of the labial palp. Five transgenic lines exhibit lacZ expression in sensory neurons of the labial sensilla (Figure 3). The expression of each transgene is restricted to a single row of chemosensory bristles. *Gr47A1*, for example, is expressed in sensilla innervating the most peripheral row of bristles, whereas *Gr66C1* is expressed in sensilla that occupy the most medial column (Figure 3). Flies bearing a *GR promoter-Gal4* gene were also crossed with *UAS-GFP* stocks. The expression of GFP allows greater cellular definition and reveals that each receptor is expressed in a single neuron within a sensillum (Figure 4A, 4B). The pattern of GR gene expression determined by GR promoter transgenes resembles that seen by *in situ* hybridization. However, co-expression of the transgene reporter and the endogenous gene could not be directly demonstrated by dual label *in situ* hybridization due to low levels of GR gene expression. Nevertheless, this pattern of expression, in which a receptor is expressed in only one neuron in a sensillum and in one sensillar row, is maintained in over 50 individuals examined for each transgenic line and is also maintained in independent transformed lines for each GR transgene.

#### **Receptor Expression in Other Chemosensory Neurons**

Chemosensory bristles reside at multiple anatomic sites in the fly including the taste organs in the mouth, the legs and wings, as well as in the female genitalia (Table 1)



(Stocker, 1994). Three sensory organs reside deep in the mouth: the labral sense organ (comprised of 10 chemosensory neurons) and the ventral and dorsal cibarial organs (each containing six chemosensory neurons) (Stocker and Schorderet, 1981; Nayak and Singh, 1983). The function of these specialized sensory organs is unknown, but their anatomic position and CNS projection pattern suggests that they participate in taste recognition (Stocker and Schorderet, 1981; Nayak and Singh, 1983). Three of the five *GR promoter-Gal4* lines that are expressed in the proboscis are also expressed in the cibarial organs (Figure 4C; Table 1). One gene, *Gr2B1*, is expressed solely in the labral sense organ and is not detected in the proboscis labellum or in the cibarial organs (Figure 4D).

Chemosensory bristles also decorate both the legs and wings of *Drosophila* with about 40 chemosensory hairs on each structure (Nayak and Singh, 1983; Hartenstein and Posakony, 1989). One gene, *Gr32D1*, expressed both in the proboscis and cibarial organ, is also expressed in two to three neurons in the most distal tarsal segments of all legs (Figure 4E). These results are consistent with the observation that exposure of the legs to tastants results in proboscis extension and feeding behavior (Dethier, 1976). The observation that members of this gene family are expressed in the proboscis and in chemosensory cells of the internal mouth organs and leg suggests that this gene family encodes gustatory receptors.

#### **Expression of Gustatory Receptors in *Drosophila* Larvae**

The expression of GR transgenes in larvae was also examined. The detection of food in larvae is mediated by chemosensors that reside largely in the antennal-maxillary

complex, a bilaterally symmetric anterior structure composed of the dorsal and terminal organs (Figure 5A; Table 1) (Stocker, 1994; Campos-Ortega and Hartenstein, 1997; Heimbeck et al., 1999). Each of the two larval chemosensory organs comprises about 40 neurons. Neurons of the dorsal organ primarily detect volatile odorants (Stocker, 1994), whereas the terminal organ is thought to detect both soluble and volatile chemical cues (Heimbeck et al., 1999).

The possibility that members of the GR family are expressed in larval chemosensory cells was addressed by examining the larval progeny that result from crosses between *GR promoter-Gal4* and *UAS-GFP* flies. Examination of live larvae by direct fluorescent microscopy reveals that five of the seven GRs expressed in the adult are expressed in single neurons within the terminal organ (Figure 5 and Table 1). GR-promoter fusions from each of the 5 genes show bilateral expression of GFP both in the neuronal cell body and in the dendrite. The dendrites extend anteriorly to terminate in the terminal organ, a dome-shaped structure that opens to the environment. In about 5% of the larvae, a second positive cell is observed in each of the lines.

*Gr2B1* is expressed in only a single neuron in the labral sense organ of the adult, but is expressed in an extensive population of chemosensory cells in larvae. This gene is expressed in two neurons innervating the dorsal organ, one neuron innervating the terminal organ, and a single bilaterally symmetric neuron innervating the ventral pit in each thoracic hemisegment (Figure 5C). The ventral pit contains a single sensory neuron that may be involved in contact chemosensation. The GR genes are therefore likely

to play a significant role in chemosensory recognition in larvae as well as adults.

### **The Diversity of GR expression in Individual Neurons**

Olfactory neurons of mammals as well as *Drosophila* express a single odorant receptor such that the brain can discriminate odor by determining which neurons have been activated (Ngai et al., 1993; Ressler et al., 1993; Vassar et al., 1993; Chess et al., 1994; Gao et al., 2000; Vosshall et al., 2000). In contrast, nematode olfactory neurons and mammalian gustatory cells co-express multiple receptor genes (Bargmann and Horvitz, 1991; Troemel et al., 1995; Hoon et al., 1999; Adler et al., 2000). The diversity of GR gene expression in individual larval taste neurons was therefore examined. In larvae, most receptors are expressed in only one neuron in the terminal organ. Crosses between five *GR promoter-Gal4* lines and flies bearing *UAS-GFP* reveal a single intensely stained neuron within each terminal organ. Seven lines bearing two different *GR promoter-Gal4* transgenes along with the *UAS-GFP* reporter were then generated. In every line bearing two *GR promoter-Gal4* fusions, two GFP positive cells per terminal organ were observed (Figure 5F, 5G). These experiments demonstrate that individual gustatory neurons of larvae express different complements of receptors and are likely to respond to different chemosensory cues.

### **The Projections of Larval Chemosensory Neurons to the Brain**

In other sensory systems, a spatial map of receptor activation in the periphery is maintained in the brain such that the quality of a sensory stimulus may be encoded in spatially defined patterns of neural activity. *GR promoter-Gal4* transgenes were therefore used to drive the

expression of *UAS-nSyb-GFP* to visualize the projections of sensory neurons expressing different GR genes. nSyb-GFP is a C-terminal fusion of green fluorescent protein to neuronal synaptobrevin that selectively labels synaptic vesicles, allowing the visualization of terminal axonal projections (Estes et al., 2000). Whole mount brain preparations from transgenic flies were examined by immunofluorescence with an antibody against GFP and a monoclonal antibody, nc82, which labels neuropil and identifies the individual glomeruli in the antennal lobe (Laissue et al., 1999). These experiments were initially performed with larvae because of the relative simplicity of the larval brain and the observation that a given GR is expressed in only a small number of gustatory neurons.

The *Drosophila* larval brain is composed of two dorsal brain hemispheres fused to the ventral hindbrain (Figure 6A). The brain hemispheres and the hindbrain contain an outer shell of neuronal cell bodies and a central fibrous neuropil. Determination of the number of neuroblasts and the number of cell divisions suggest that there are approximately 10,000-15,000 neurons in the larval brain, a value 10-20 fold lower than in the adult (Hartenstein and Campos-Ortega, 1984; Hartenstein et al., 1987; Truman et al., 1993). Chemosensory neurons send axonal projections to two distinct regions of the larval brain, the antennal lobe and the subesophageal ganglion (SOG) (Stocker, 1994; Heimbeck, et al., 1999). The antennal lobe is a small neuropil in the medial aspect of the deutero-cerebrum within each brain hemisphere. The antennal lobe receives input from neurons of the dorsal and terminal organ and presumably participates in processing olfactory information. The SOG resides in the most anterior aspect of the hindbrain, at the juncture of the hindbrain with

the brain hemispheres. The SOG receives input from the terminal organ and mouthparts and is thought to process gustatory information. Whereas the projections of populations of chemosensory cells have been traced to the antennal lobe and the SOG, the patterns of axonal projections for individual sensory cells have not been described. Moreover, the connections of chemosensory axons with second order brain neurons is unknown for the larval brain.

*Gr32D1-Gal4* is expressed in multiple neurons in the proboscis of the adult, but it is expressed in only a single neuron in the terminal organ of larvae (Figure 5B). In larvae containing the *Gr32D1-Gal4* and *UAS-nSyb-GFP* transgenes, it is possible to visualize the axons of *Gr32D1*-expressing cells as they course posteriorly to enter the subesophageal ganglion (data not shown). The axons then turn dorsally and intensely stained fibers terminate in the medial aspect of the SOG (Figure 6C). A similar pattern is observed for neurons expressing *Gr66C1* (Figure 6B,D), a gene expressed in the proboscis in the adult and in a single neuron in the terminal organ and two in the mouth of larvae (Figure 5E). However, the terminal arbors of *Gr66C1* neurons are consistently thicker than that observed for *Gr32D1*, perhaps reflecting the increased number of *Gr66C1*-bearing neurons. The reporter *nSyb-GFP* stains axons only weakly but shows intense staining of what is likely to be terminal projections of sensory neurons that synapse on second order neurons in the neuropil of the SOG. This terminal arbor extends for about 40  $\mu$ m and reveals a looser, more distributed pattern than the tight neuropil of the olfactory glomerulus. The position and pattern of the terminal projections from individual chemosensory cells in the terminal organ show

bilateral symmetry and are maintained in over 20 larvae examined.

A more complex pattern of projections is observed for *Gr2B1*, a gene expressed in one neuron in the terminal organ, two in the dorsal organ, and a single bilaterally symmetric neuron in each thoracic hemisegment (Figure 5C). One set of fibers appears to terminate in the antennal lobe (Figure 6E). A second more posterior set of fibers can be traced from the thorax into the hindbrain, with fibers terminating posterior to the antennal lobe (Figure 6E). This pattern of projections is of interest for it implies that neurons in different locations in larvae that express the same receptor project to discrete locations in the larval brain, suggesting the possibility that the same chemosensory stimulus can elicit distinct behavioral outputs.

An attempt was made to determine whether neurons in the terminal organ that express different GRs project to discrete loci within the SOG. Larvae that express two promoter fusions, *Gr66C1-Gal4* and *Gr32D1-Gal4*, along with a *UAS-nSyb-GFP* transgene were generated. The projections in these flies are broadened, suggesting that these sets of neurons terminate in overlapping but non-identical regions of the SOG (Figure 6F). More definitive data to support the existence of a topographic map of taste quality will require two color labelling of the different fibers to discern whether the projections from neurons expressing different GRs are spatially segregated in the SOG.

#### **Are GRs also Odorant Receptors?**

A large family of presumed olfactory receptor genes in

*Drosophila* (the DOR genes) has been identified that is distinct from the GR gene family (Clyne et al., 1999; Gao and Chess, 1999; Vosshall et al., 1999; Vosshall et al., 2000). Expression of the DOR genes is only observed in olfactory sensory neurons within the antenna and maxillary palp, where a given DOR gene is expressed in a spatially invariant subpopulation of cells (Clyne et al., 1999; Gao and Chess, 1999; Vosshall et al., 1999; Vosshall et al., 2000). *In situ* hybridization experiments demonstrate that three members of the GR gene family are also expressed in subpopulations of antennal neurons (Figure 2B). These observations suggest either that the odorant receptors in *Drosophila* are encoded by at least two different gene families or that previously unidentified taste responsive neurons reside within the antenna.

In *Drosophila*, olfactory information is transmitted to the antennal lobe, whereas gustatory neurons in the proboscis and mouth relay sensory information to the subesophageal ganglion (Stocker, 1994). The spatial pattern of expression of GRs in the antenna and the pattern of projections of their sensory axons in the brain were therefore examined. *In situ* hybridization with the three GR genes reveals that each gene is expressed in about 20-30 cells/gene in the antenna (Figure 2B). Similar results are obtained in a cross between an antennal GR promoter-Gal4 line, Gr21D1-Gal4, and UAS-LacZ or UAS-GFP lines (Figure 7A, 7B). This pattern of GR gene expression is maintained in over 50 antennae that have been analyzed. The GR-positive cells occupy regions of the antenna that do not express identified members of the DOR gene family (Vosshall et al., 2000), suggesting that there is spatial segregation of these two receptor families.

Whether antennal neurons expressing a GR gene project to the antennal lobe in a manner analogous to that observed for cells expressing the DOR genes was next addressed. Transgenic flies expressing a *Gr21D1* promoter-*Gal4* fusion were crossed to animals bearing the *UAS-nSyb-GFP* transgene. These studies demonstrate that neurons expressing the *Gr21D1* transgene project to a single, bilaterally symmetric glomerulus in the ventral-most region of the antennal lobe (the V glomerulus) (Figure 7C) (Stocker et al., 1990; Laissue et al., 1999) and do not project to the SOG. Thus, as in the case of the family of DOR genes (Gao et al., 2000; Vosshall et al., 2000), neurons expressing the same receptor project to a single spatially invariant glomerulus.

*Gr21D1* is also expressed in one cell of the terminal organ of larvae (Figure 5D). The projections of *Gr21D1*-bearing neurons were therefore traced to the larval brain. *Gr21D1* axons enter the larval brain and terminate in the antennal lobe rather than the SOG (Figure 6G). The segregation of projections from presumed olfactory and gustatory neurons is apparent in larvae that contain *Gr21D1-Gal4* and *Gr66C1-Gal4* along with *UAS-nSyb-GFP*. In these transgenic flies, two distinct sets of termini are observed, one entering the SOG, and a second entering the antennal lobe (Figure 6H).

Thus, a member of the GR gene family is expressed in sensory neurons of the antenna and the terminal organ of larvae, and GR-bearing neurons project to the antennal lobe. These data indicate that at least two independent gene families, the DORs and the GRs, recognize olfactory information. The GR gene family is therefore likely to encode both olfactory and gustatory receptors, and neurons



expressing distinct classes of GR receptors project to different regions of the fly brain.

10081815.02202

**Table 1. Summary of *Drosophila* chemosensory tissues and GR transgene expression patterns.**

The table summarizes the expression patterns of GR promoter-Gal4 transgenes in adult and larval chemosensory tissues. Adult *Drosophila* sense gustatory cues with chemosensory bristles on the labellum of the proboscis, legs and wings, and with specialized structures of the internal mouthparts, the cibarial organs and the labral sense organ. Gustatory neurons on the proboscis send axonal projections to the subesophageal ganglion (SOG). Sensory neurons on the antenna recognize olfactory cues and project to the antennal lobe (AL). In *Drosophila* larvae, gustatory cues are recognized by neurons innervating the terminal organ and possibly the ventral pits, and olfactory cues are recognized by neurons innervating the dorsal organ and the terminal organ. Gustatory tissues are highlighted in blue and olfactory tissues are highlighted in pink. The schematic of the adult fly is adapted from Stocker (1994). The schematic of the larva is adapted from Struhl (1981).

Table 1: Expression profiles of GR transgenes

ADULT			LARVA								
GR	In situ signal	labellum	antenna	cibarial organs	labral organ	leg	terminal organ	dorsal organ	mouth	gut	ventral pits
Gr2B1	-	-	-	-	+	-	+	+	-	+	+
Gr21D1	antenna	-	+	-	-	-	+	-	-	-	-
Gr22B1	-	+	-	-	-	-	-	-	-	-	-
Gr28A1	labellum	+	-	+	-	-	+	-	+	-	-
Gr32D1	labellum	+	-	+	-	+	+	-	-	-	-
Gr47A1	labellum	+	-	-	-	-	-	-	-	-	-
Gr66C1	labellum	+	-	+	-	-	+	-	+	-	-

## DISCUSSION

### A Family of Gustatory and Olfactory Receptors

Specialized sense organs have evolved to recognize chemosensory information in the environment. The antennae in insects, the amphid in nematodes, and the nose of mammals allow the recognition of a vast repertoire of volatile odorants often over long distances. Taste organs have evolved to accommodate a distinct function, the recognition of soluble chemical cues over shorter distances. In vertebrates, taste is largely restricted to the tongue and palate, whereas in insects, gustatory neurons are more broadly distributed along the body plan and reside not only in the proboscis and pharynx but also on the wings, legs, and female genitalia. Anatomic and functional segregation of the gustatory and olfactory systems is not only apparent in the peripheral receptor field but in the projections to the brain. In the fly, for example, olfactory neurons project to the antennal lobe, whereas most gustatory neurons ultimately synapse within the subesophageal ganglion. This separation is also observed in vertebrates where taste and smell are accommodated by distinct sense organs and conveyed to different brain regions by different cranial nerves. Thus, a common sensory function, the recognition of chemical cues, has undergone specialization to allow for the recognition of at least two distinct categories of chemosensory information, each eliciting distinct behavioral responses.

This study has characterized the patterns of expression of a large family of genes in *Drosophila* that are likely to encode both odorant and gustatory receptors. A family of candidate taste receptors was identified by searching the *Drosophila* genome with an algorithm designed to detect

genes encoding seven transmembrane domain proteins (Clyne et al., 2000). This analysis was extended through a search of the complete euchromatic genome of *Drosophila* and identify 56 genes within the family. All of the GR genes contain a signature motif in the carboxyl terminus that is also present within some members of the DOR gene family, suggesting that these two families share a common origin.

The GR family of proteins was tentatively identified as gustatory receptors solely on the basis of PCR analysis of proboscis RNA (Clyne et al., 2000). *In situ* hybridization and transgene experiments demonstrate that members of this gene family are expressed in the antennae, proboscis, pharynx, leg, and larval chemosensory organs. Thus, a single gene family encodes chemosensory receptors containing both olfactory and gustatory receptors. Flies bearing GR promoter transgenes were generated from 15 GR genes. Expression is observed in seven lines and is restricted to chemosensory cells. No expression is detected in other neurons or in non-neuronal cells. These data suggest that the expression of this family is limited to gustatory and olfactory neurons, and that the inability to observe expression in 8 transgenic lines perhaps reflects the structural inadequacy of the promoters.

A common gene family encoding both olfactory and taste receptors is not present in vertebrates where the main olfactory epithelium, the vomeronasal organ and the tongue express receptors encoded by independent gene families (Buck and Axel, 1991; Dulac and Axel, 1995; Herrada and Dulac, 1997; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997; Hoon et al., 1999; Adler et al., 2000; Matsunami et al., 2000). The observations described

herein are more reminiscent of the chemosensory receptor families in *C. elegans* that encode odorant receptors expressed in the amphid neurons and taste receptors in sensory neurons responsive to soluble chemicals (Troemel et al., 1995; Troemel, 1999).

#### **Patterns of GR Gene Expression and Taste Modalities**

The size of the family of candidate taste receptors and the pattern of expression in chemosensory cells provides insight into the problem of the recognition and discrimination of gustatory cues. On average, each GR is expressed in 5% of the cells in the proboscis labellum, suggesting that the proboscis alone will contain at least 20 distinct taste cells expressing about 20 different GR receptors. Moreover, a given receptor is expressed in one of the four rows of sensilla such that the sensilla in different rows are likely to be functionally distinct. Electrophysiologic studies have suggested that all sensilla are identical and contain four distinct cells each responsive to a different category of taste (Dethier, 1976; Rodrigues and Siddiqi, 1978; Fujishiro et al., 1984). The data presented herein are not consistent with these conclusions and argue that different rows of sensilla are likely to contain cells with different taste specificities.

At present the nature of the ligands recognized by these GR receptors are not known, nor is it known whether all taste modalities are recognized by this gene family. In mammals, gustatory cues have classically been grouped into five categories: sweet, bitter, salt, sour and glutamate (*umami*) (Kinnamon and Margolskee, 1996; Lindemann, 1996; Gilbertson et al., 2000). Sugar and bitter taste are likely to be mediated by G protein-coupled receptors since

these modalities require the function of a taste cell-specific  $G_a$  subunit, gustducin (McLaughlin et al., 1992; Wong et al., 1996). Recently, two novel families of seven transmembrane proteins (the T1Rs and T2Rs) were shown to be selectively expressed in taste cells in the tongue and palate epithelium (Hoon et al., 1999; Adler et al., 2000; Matsunami et al., 2000). Genetic experiments implicated members of the T2R family in the recognition of bitter tastants (Adler et al., 2000; Matsunami et al., 2000) and functional studies directly demonstrated that members of the T2R family serve as gustducin-linked bitter taste receptors. (Chandrashekar et al., 2000). A large number of candidate genes have been suggested to encode receptors for other taste modalities but in only a few instances have functional data and expression patterns supported these assumptions. In mammals, an amiloride-sensitive sodium channel has been suggested as the salt receptor (Heck et al., 1984), a degenerin homolog (MDEG-1) (Ugawa et al., 1998) and a potassium channel (Kinnamon et al., 1988) as sour or pH sensors, and a rare splice form of the metabotropic glutamate receptor as the *umami* sensor (Chaudhari et al., 2000). In *Drosophila*, genetic analysis of mutant flies defective in the recognition of the sugar, trehalose, has led to the identification of a transmembrane receptor distinct from GRs that reduces the sensitivity to one class of sugars (Ishimoto et al., 2000). The interpretation of the role of these putative taste receptors in taste perception awaits a more definitive association between tastant and gene product.

### **The Logic of Taste Discrimination**

How does the fly discriminate among multiple tastants? One mechanism of chemosensory discrimination, thought to operate in the olfactory system of insects and

vertebrates, requires that individual sensory neurons express only one of multiple receptor genes (Buck and Axel, 1991; Ngai et al., 1993; Ressler et al., 1993; Vassar et al., 1993; Chess et al., 1994; Clyne et al., 1999; Gao and Chess, 1999; Vosshall et al., 1999). Neurons expressing a given receptor project axons that converge on topographically invariant glomeruli such that different odors elicit different patterns of spatial activity in the brain (Ressler et al., 1994; Vassar et al., 1994; Mombaerts et al., 1996; Wang et al., 1998; Gao et al., 2000; Vosshall et al., 2000). The nematode *C. elegans* uses a rather different logic, in which a given sensory neuron dictates a specific behavior but expresses multiple receptors (Bargmann and Horvitz, 1991; Troemel et al., 1995; Troemel et al., 1997). In the worm olfactory system, discrimination is necessarily more limited and exploits mechanisms to diversify the limited number of sensory cells (Colbert and Bargmann, 1995; Troemel et al., 1999; L'Etoile and Bargmann, 2000). A similar logic has been suggested for mammalian taste. Several members of the T2R family of about 50 receptor genes, each thought to encode bitter sensors, are co-expressed in sensory cells within the tongue (Adler et al., 2000). This organization allows the organism to recognize a diverse repertoire of aversive tastants but limits the ability to discriminate among them.

What can be discerned about the logic of taste discrimination from the pattern of GR gene expression in *Drosophila*? First, the number of GR genes, 56, approximates the number of DOR genes, suggesting that the fly recognizes diverse repertoires of both soluble and volatile chemical cues. Moreover, the data presented herein argue that individual sensory neurons differ with



respect to receptor gene expression and are therefore functionally distinct. Experiments with *Drosophila* larvae demonstrate that a given GR gene is expressed in one neuron in the larval terminal organ. Strains bearing two different GR-promoter fusions reveal twice the number of expressing cells. Similar results are obtained in adult gustatory organs (data not shown). More definitive experiments to examine the diversity of receptor expression in a single neuron, employed successfully in the olfactory system, have been difficult since the levels of GR RNA are 10-20 fold lower than odorant receptor RNA levels. Nevertheless, experiments described herein demonstrate that different gustatory neurons express different complements of GR genes and at the extreme are consistent with a model in which gustatory neurons express only a single receptor gene.

How does the brain discern which of the different gustatory neurons is activated by a given tastant? As in other sensory systems, it is possible that axons from different taste neurons segregate to spatially distinct loci in the subesophageal ganglion. In such a model, taste quality would be represented by different spatial patterns of activity in the brain. Preliminary experiments suggest that neurons expressing different GRs project to spatially segregated loci within the brain. Clear segregation of axonal termini is observed for presumed taste neurons that project to the SOG and olfactory neurons that project to the antennal lobe. A second interesting pattern of projections is observed for the presumed gustatory receptor *Gr2B1*, a gene expressed in neurons in the terminal and dorsal organs and in a single neuron in the ventral pit present bilaterally in each thoracic segment. At least two spatially segregated

10081815-022202

targets are observed for these neurons in the larval brain: one set of fibers terminates in glomeruli of the antennal lobe and a second set of fibers (from the ventral pits) project to the SOG. Thus, neurons expressing the same receptor in different chemosensory organs project to distinct brain regions. In this manner, the same chemosensory cue could elicit distinct behaviors depending upon the cell it activates. Sucrose, for example, could elicit chemoattraction upon exposure to the thoracic neurons and eating behavior upon activation of neurons in the terminal and dorsal organ.

These data establish that presumed olfactory neurons and gustatory neurons expressing GR genes project to different regions of the larval brain. Taste neurons expressing different GR genes, however, all project to the SOG. The current data do not permit us to discern whether axons from neurons expressing different GR genes project to spatially distinct loci within the SOG. The axon termini of gustatory neurons terminate in more diffuse, elongated structures than the tightly compacted glomeruli formed by olfactory sensory axons, rendering it difficult at present to discern a topographic map of gustatory projections in the larval brain.

### **Sensory Perception in Larvae**

Insects provide an attractive model system for the study of chemosensory perception because they exhibit sophisticated taste and olfactory driven behaviors that are controlled by a chemosensory system that is anatomically and genetically simpler than vertebrates (Nassif et al., 1998). *Drosophila* larvae afford a particularly facile organism because much of their behavior surrounds eating. Gustatory neurons in the

10081816-02202

terminal organ and along the body plan, together with olfactory sensory cells in the dorsal and terminal organs, combine to identify food sources and elicit eating behaviors (Stocker, 1994).

Members of the *Drosophila* odorant receptor (DOR) family are expressed in the adult olfactory system but cannot be detected in larval chemosensory organs. GR genes are expressed in larval olfactory and gustatory neurons and may encode the entire repertoire of larval chemosensory receptors. The simplicity of the *Drosophila* larvae, coupled with the ease of behavioral studies, suggests that it may be possible to relate the recognition of chemosensory information to specific behavioral responses and ultimately to associate changes in behavior with modifications in specific connections.

## References

- Adams, M. et al. (2000). The genome sequence of *Drosophila melanogaster*. *Science* 287, 2185-2195.
- Adler, E., Hoon, M.A., Mueller, K.L., Chandrashekar, J., Ryba, N.J. and Zuker, C.S. (2000). A novel family of mammalian taste receptors. *Cell* 100, 693-702.
- Altschul, S., Madden, T., Schaffer, A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res* 25, 3389-402.
- Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990). Basic local alignment search tool. *J. Mol. Biol.* 215, 403-410.
- Arora, K., Rodrigues, V., Joshi, S., Shanbhag, S. and Siddiqi, O. (1987). A gene affecting the specificity of the chemosensory neurons of *Drosophila*. *Nature* 330, 62-63.
- Bargmann, C.I. and Horvitz, H.R. (1991). Chemosensory neurons with overlapping functions direct chemotaxis to multiple chemicals in *C. elegans*. *Neuron* 7, 729-742.
- Brand, A.H. and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 118, 401-415.
- Buck, L. and Axel, R. (1991). A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* 65, 175-187.

Campos-Ortega, J.A. and Hartenstein, V. (1997). The embryonic development of *Drosophila Melanogaster*. Berlin, Springer.

Chandrashekar, J., Mueller, K.L., Hoon, M.A., Adler, E., Feng, L., Guo, W., Zuker, C.S. and Ryba, N.J. (2000). T2Rs function as bitter taste receptors. *Cell* 100, 703-711.

Chaudhari, N., Landin, A.M. and Roper, S.D. (2000). A metabotropic glutamate receptor variant functions as a taste receptor. *Nat. Neurosci.* 3, 113-119.

Chess, A., Simon, I., Cedar, H. and Axel, R. (1994). Allelic inactivation regulates olfactory receptor gene expression. *Cell* 78, 823-834.

Clyne, P.J., Warr, C.G. and Carlson, J.R. (2000). Candidate Taste Receptors in *Drosophila*. *Science* 287, 1830-1834.

Clyne, P.J., Warr, C.G., Freeman, M.R., Lessing, D., Kim, J. and Carlson, J.R. (1999). A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in *Drosophila*. *Neuron* 22, 327-338.

Colbert, H.A. and Bargmann, C.I. (1995). Odorant-specific adaptation pathways generate olfactory plasticity in *C. elegans*. *Neuron* 14, 803-812.

Dethier, V.G. (1976). *The Hungry Fly*. Cambridge, MA, Harvard University Press.

Dulac, C. and Axel, R. (1995). A novel family of genes encoding putative pheromone receptors in mammals. *Cell* 83, 195-206.

Eddy, S.R. (1998). Profile hidden Markov models. *Bioinformatics* 14, 755-763.

Estes, P.E., Ho, G., Narayanan, R. and Ramaswami, M. (2000). Synaptic localization and restricted diffusion of a *Drosophila* neuronal synaptobrevin - green fluorescent protein chimera in vivo. *J. Neurogenetics* 13, 233-255.

Falk, R., Bleiser-Avivi, N. and Atidia, J. (1976). Labellar Taste Organs of *Drosophila Melanogaster*. *Journal of Morphology* 150, 327-341.

Fujishiro, N., Kijima, H. and Morita, H. (1984). Impulse frequency and action potential amplitude in labellar chemosensory neurons of *Drosophila Melanogaster*. *Journal of Insect Physiology* 30, 317-325.

Gao, Q. and Chess, A. (1999). Identification of candidate *Drosophila* olfactory receptors from genomic DNA sequence. *Genomics* 60, 31-39.

Gao, Q., Yuan, B. and Chess, A. (2000). Convergent Projections of *Drosophila* Olfactory Neurons to Specific Glomeruli in the Antennal Lobe. *Nature Neurosci.* 3, 780-785.

Gilbertson, T.A., Damak, S. and Margolskee, R.F. (2000). The molecular physiology of taste transduction. *Curr Opin Neurobiol* 10, 519-527.

Hartenstein, V. and Campos-Ortega, J.A. (1984). Early neurogenesis in wild-type *Drosophila melanogaster*. Wilhelm Roux's Arch Dev Bio 193, 308-325.

Hartenstein, V. and Posakony, J.W. (1989). Development of adult sensilla on the wing and notum of *Drosophila melanogaster*. Development 107, 389-405.

Hartenstein, V., Rudloff E. and Campos-Ortega, J.A. (1987). The pattern of proliferation of the neuroblasts in the wild-type embryo of *Drosophila melanogaster*. Wilhelm Roux's Arch Dev Bio 198, 264-274.

Heck, G.L., Mierson, S. and DeSimone, J.A. (1984). Salt taste transduction occurs through an amiloride-sensitive sodium transport pathway. Science 223, 403-405.

Heimbeck, G., Bugnon, V., Gendre, N., Haberlin, C. and Stocker, R.F. (1999). Smell and taste perception in *Drosophila melanogaster* larva: toxin expression studies in chemosensory neurons. J. Neurosci. 19, 6599-6609.

Herrada, G. and Dulac, C. (1997). A novel family of putative pheromone receptors in mammals with a topographically organized and sexually dimorphic distribution. Cell 90, 763-773.

Higgins, D.G. and Sharp, P.M. (1988). CLUSTAL: a package for performing multiple sequence alignment on a microcomputer. Gene 73, 237-244.

Hoon, M.A., Adler, E., Lindemeier, J., Battey, J.F., Ryba, N.J. and Zuker, C.S. (1999). Putative mammalian taste

receptors: a class of taste-specific GPCRs with distinct topographic selectivity. *Cell* 96, 541-551.

Ishimoto, H., Matsumoto, A. and Tanimura, T. (2000). Molecular identification of a taste receptor gene for trehalose in *Drosophila*. *Science* 289, 116-119.

Kinnamon, S.C., Dionne, V.E. and Beam, K.G. (1988). Apical localization of K<sup>+</sup> channels in taste cells provides the basis for sour taste transduction. *Proc. Natl. Acad. Sci. U S A* 85, 7023-7027.

Kinnamon, S.C. and Margolskee, R.F. (1996). Mechanisms of taste transduction. *Curr. Opin. Neurobiol.* 6, 506-513.

Kunishima, N., Shimada, Y., Tsuji, Y., Sato, T., Yamamoto, M., Kumasaka, T., Nakanishi, S., Jingami, H. and Morikawa, K. (2000). Structural basis of glutamate recognition by a dimeric metabotropic glutamate receptor. *Nature* 407, 971-977.

Laissue, P.P., Reiter, C., Hiesinger, P.R., Halter, S., Fischbach, K.F. and Stocker, R.F. (1999). Three-dimensional reconstruction of the antennal lobe in *Drosophila melanogaster*. *J. Comp. Neurol.* 405, 543-552.

L'Etoile, N.D. and Bargmann, C.I. (2000). Olfaction and Odor Discrimination Are Mediated by the *C. elegans* Guanylyl Cyclase ODR-1. *Neuron* 25, 575-586.

Lindemann, B. (1996). Taste reception. *Physiol. Rev.* 76, 718-766.

20220916 09:00



Matsunami, H. and Buck, L.B. (1997). A multigene family encoding a diverse array of putative pheromone receptors in mammals. *Cell* 90, 775-784.

Matsunami, H., Montmayeur, J.P. and Buck, L.B. (2000). A family of candidate taste receptors in human and mouse. *Nature* 404, 601-604.

McLaughlin, S.K., McKinnon, P.J. and Margolskee, R.F. (1992). Gustducin is a taste-cell-specific G protein closely related to the transducins. *Nature* 357, 563-569.

Mombaerts, P., Wang, F., Dulac, C., Chao, S.K., Nemes, A., Mendelsohn, M., Edmondson, J. and Axel, R. (1996). Visualizing an olfactory sensory map. *Cell* 87, 675-686.

Nassif, C., Noveen, A. and Hartenstein, V. (1998). Embryonic Development of the *Drosophila* Brain. I. Pattern of Pioneer Tracts. *J. Comp. Neurol.* 402, 10-31.

Nayak, S.V. and Singh, R.N. (1983). Sensilla on the tarsal segments and mouthparts of adult *Drosophila Melanogaster*. *International Journal of Insect Morphology and Embryology* 12, 273-291.

Ngai, J., Chess, A., Dowling, M.M., Necles, N., Macagno, E.R. and Axel, R. (1993). Coding of olfactory information: topography of odorant receptor expression in the catfish olfactory epithelium. *Cell* 72, 667-680.

Possidente, D.R. and Murphey, R.K. (1989). Genetic control of sexually dimorphic axon morphology in *Drosophila* sensory neurons. *Dev. Biol.* 132, 448-457.

Power, M.E. (1948). The thoracico-abdominal nervous system of an adult insect, *Drosophila Melanogaster*. *Journal of Comparative Neurology* 88, 347-409.

Rajashekhar, K.P. and Singh, R.N. (1994). Neuroarchitecture of the tritocerebrum of *Drosophila melanogaster*. *J. Comp. Neurol.* 349, 633-645.

Ray, K., Hartenstein, V. and Rodrigues, V. (1993). Development of the taste bristles on the labellum of *Drosophila melanogaster*. *Dev. Biol.* 155, 26-37.

Ressler, K.J., Sullivan, S.L. and Buck, L.B. (1993). A zonal organization of odorant receptor gene expression in the olfactory epithelium. *Cell* 73, 597-609.

Ressler, K.J., Sullivan, S.L. and Buck, L.B. (1994). Information coding in the olfactory system: evidence for a stereotyped and highly organized epitope map in the olfactory bulb. *Cell* 79, 1245-1255.

Rice, M.J. (1977). Blowfly ovipositor receptor neurons sensitive to monovalent cation concentration. *Nature* 268, 747-749.

Rodrigues, V. and Siddiqi, O. (1978). Genetic analysis of a chemosensory pathway. *Proceedings of the Indian Academy of Science, Series B* 87, 147-160.

Rubin, G.M., Hazelrigg, T., Karess, R.E., Laski, F.A., Laverty, T., Levis, R., Rio, D.C., Spencer, F.A. and Zuker, C.S. (1985). Germ line specificity of P-element transposition and some novel patterns of expression of

transduced copies of the white gene. Cold Spring Harb. Symp. Quant .Biol. 50, 329-335.

Rubin, G.M., et al. (2000). Comparative genomics of the eukaryotes. Science 287, 2204-2215.

Ryba, N.J. and Tirindelli, R. (1997). A new multigene family of putative pheromone receptors. Neuron 19, 371-379.

Saitou, N. and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Bio. Evol. 4, 406-425.

Shanbhag, S.R. and Singh, R.N. (1992). Functional implications of the projections of neurons from individual labellar sensillum of *Drosophila Melanogaster* as revealed by neuronal marker horseradish peroxidase. Cell and Tissue Research 267, 273-282.

Singh, R.N. (1997). Neurobiology of the gustatory systems of *Drosophila* and some terrestrial insects. Microsc. Res. Tech. 39, 547-563.

Stocker, R.F. (1994). The organization of the chemosensory system in *Drosophila melanogaster*: a review. Cell Tissue Res. 275, 3-26.

Stocker, R.F., Lienhard, M.C., Borst, A. and Fischbach, K.F. (1990). Neuronal architecture of the antennal lobe in *Drosophila melanogaster*. Cell Tissue Res. 262, 9-34.

Stocker, R.F. and Schorderet, M. (1981). Cobalt filling of sensory projections from internal and external mouthparts in *Drosophila*. *Cell Tissue Res.* 216, 513-523.

Struhl, G. (1981). A gene product required for correct initiation of segmental determination in *Drosophila*. *Nature* 293, 36-41.

Taylor, B.J. (1989). Sexually dimorphic neurons of the terminalia of *Drosophila melanogaster*: II. Sex-specific axonal arborizations in the central nervous system. *J. Neurogenet.* 5, 193-213.

Tompkins, L., Siegel, R.W., Gailey, D.A. and Hall, J.C. (1983). Conditioned courtship in *Drosophila* and its mediation by association of chemical cues. *Behav. Genet.* 13, 565-578.

Troemel, E.R. (1999). Chemosensory signaling in *C. elegans*. *Bioessays* 21, 1011-1020.

Troemel, E.R., Chou, J.H., Dwyer, N.D., Colbert, H.A. and Bargmann, C.I. (1995). Divergent seven transmembrane receptors are candidate chemosensory receptors in *C. elegans*. *Cell* 83, 207-218.

Troemel, E.R., Kimmel, B.E. and Bargmann, C.I. (1997). Reprogramming chemotaxis responses: sensory neurons define olfactory preferences in *C. elegans*. *Cell* 91, 161-169.

Troemel, E.R., Sagasti, A. and Bargmann, C.I. (1999). Lateral signaling mediated by axon contact and calcium entry regulates asymmetric odorant receptor expression in *C. elegans*. *Cell* 99, 387-398.

Truman, J.W., Taylor, B.J. and Awad, T.A. (1993) Formation of the adult nervous system. In The Development of *Drosophila melanogaster* Vol II, M. Bate and A.M. Arias, eds. (Cold Spring Harbor Laboratory Press), pp. 1245-1275.

Ugawa, S., Minami, Y., Guo, W., Saishin, Y., Takatsuji, K., Yamamoto, T., Tohyama, M. and Shimada, S. (1998). Receptor that leaves a sour taste in the mouth. *Nature* 395, 555-556.

Vassar, R., Chao, S.K., Sitcheran, R., Nunez, J.M., Vosshall, L.B. and Axel, R. (1994). Topographic organization of sensory projections to the olfactory bulb. *Cell* 79, 981-991.

Vassar, R., Ngai, J. and Axel, R. (1993). Spatial segregation of odorant receptor expression in the mammalian olfactory epithelium. *Cell* 74, 309-318.

Von Heijne, G. (1992). Membrane protein structure prediction, hydrophobicity analysis and the positive-inside rule. *Journal of Molecular Biology* 225, 487-494.

Vosshall, L.B., Amrein, H., Morozov, P.S., Rzhetsky, A. and Axel, R. (1999). A spatial map of olfactory receptor expression in the *Drosophila* antenna. *Cell* 96, 725-736.

Vosshall, L.B., Wong, A.M. and Axel, R. (2000). An Olfactory Sensory Map in the Fly Brain. *Cell* 102, 147-159.

Wang, F., Nemes, A., Mendelsohn, M. and Axel, R. (1998). Odorant receptors govern the formation of a precise topographic map. *Cell* 93, 47-60.

Wong, G.T., Gannon, K.S. and Margolskee, R.F. (1996).  
Transduction of bitter and sweet taste by gustducin.  
Nature 381, 796-800.

1008415-0220